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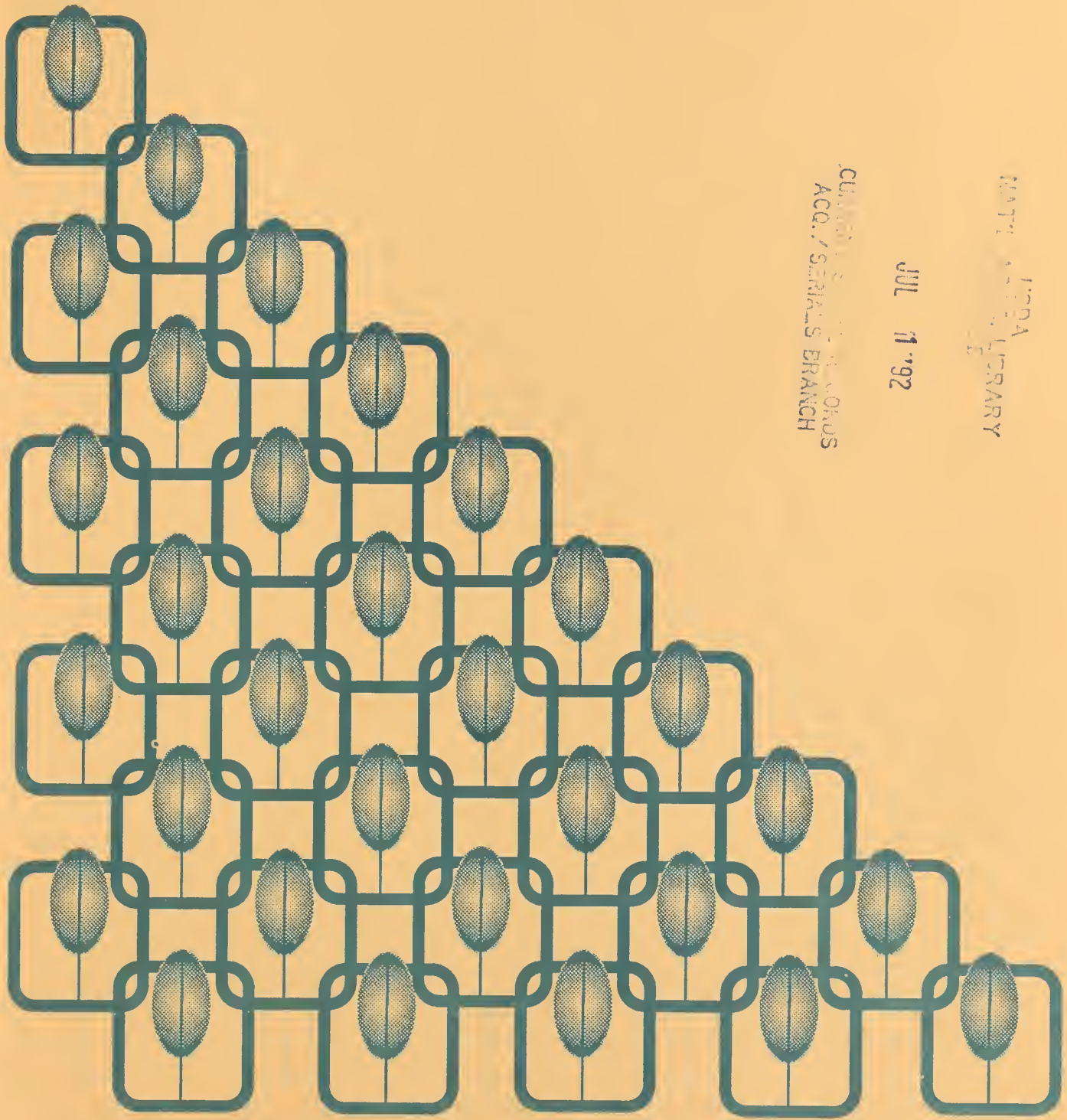

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Introduction

Apomixis is defined as "reproduction in which fertilization does not occur, so that resulting seed represents a vegetative propagation, usually of an unreduced female gamete." For several years apomixis researchers have pointed to the potential value of apomixis to the plant breeding community. For example, apomixis would make possible true-breeding hybrids varities. However, little overall attention to this area of research is evident. One reason is the lack of understanding of apomixis and how to control and move the characteristic into the important food crops.

An Apomixis Workshop held in Atlanta, Georgia on February 11-12, 1992, brought together scientists from the U.S. knowledgable in this research area. The workshop reviewed the status of apomixis knowledge and research in the U.S. and identified gaps in the present knowledge.

Twenty-one scientists representing apomixis research programs in the U.S. gave 20 minutes presentations of their current apomixis research findings and future research plans. Each speaker provided a written summary of their presentation with complete listing of references that relate to the speaker's area of apomixis research. Each speaker was limited to four pages, not including references.

The Apomixis Workshop was timely in bringing participants up to date and all participants benefitted significantly from the review of apomixis research done across many species. The remainder of this publication includes the summaries of the presentations made at the workshop followed by a summary of the future goals, objectives, and resources required for further advances in apomixis research.

Apomixis in *Cenchrus*

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Apomixis is a vegetative (asexual) method of reproduction in which the embryo develops without the union of the sperm and egg. This is a natural method for cloning plants through seed which offers a unique system for cultivar development in many species (Hanna & Bashaw, 1987). Some form of apomixis has been reported in over 300 species representing 35 plant families. The widespread occurrence of apomixis in the plant kingdom and the frequency of apomictic mechanisms in plants suggest that the basic processes responsible for asexual seed formation may be present in many wild relatives of our important food and fiber crops.

Apomixis is a common method of reproduction in perennial forage grasses and is especially prevalent in the subfamily *Panicoideae*, although it is reported in the subfamilies *Chloridoideae* and *Pooideae*. Any attempt to improve grasses in these apomictic species requires a thorough knowledge of the method of reproduction (sexual, apospory, diplospory, etc.) for each ecotype within the germplasm base to develop appropriate strategies and technology for gene transfer.

This report summarizes progress that has been made during almost 40 years of a joint research program on apomixis in grasses between the USDA-ARS Southern Crops Research Laboratory, the Texas Agricultural Experiment Station, and Texas A&M University. Much of the research during this period has concentrated on buffelgrass (*Cenchrus ciliaris* L. syn. *Pennisetum ciliare* Link.) or birdwoodgrass (*C. setigerus* Vahl.).

Taxonomically, buffelgrass and birdwoodgrass appear to be morphotypes of the same apomictic species as they are completely cross-fertile and have similar genomes (Hignight et al., 1991; Read & Bashaw, 1969). Simple allelic differences are hypothesized to account for variation in morphology and foliage color. Both species appear to be related genetically to the grassy *Pennisetum* species (n=9), but not to *Cenchrus* (sandbur). True *Cenchrus* species are sexual, have a higher base chromosome number (n=17), and are distinguished by significant basal fusion of bristles which form a "cup-like" involucre or bur surrounding the spikelets. Buffelgrass and birdwoodgrass are best classified as members of a complex of apomictic polyploids which include several species (i.e. *P. flaccidum*, *P. massaicum*, *P. mezianum*, *P. orientale*, etc.). These grassy *Pennisetum* species are not only important forage species, but are members of the tertiary gene pool of pearl millet (*P. glaucum*) and may represent germplasm for use in pearl millet breeding programs.

While plant breeding efforts continue with buffelgrass, fundamental research on the expression and regulation of apomixis now focuses on a broad range of *Cenchrus*-like and *Pennisetum* species. This agamic complex consists of morphologically similar polyploids with a base chromosome number of n=9. The predominant method of reproduction for this group is obligate nucellar apospory with pseudogamy, although sexual and facultative forms have been reported (Bashaw et al., 1992; Bray, 1978; Hignight et al., 1991; Hussey et al., 1991 and Sherwood et al., 1980).

Our experiences with buffelgrass exemplify many of the problems and opportunities which exist for improving apomictic plants. Results of early research with buffelgrass and birdwoodgrass concluded that most accessions reproduce by obligate apomixis (Fisher et al., 1954). The discovery of a sexual plant (B-1S), heterozygous for method of reproduction (MOR) (Bashaw 1962, 1969) led to the development of 1) a genetic model which described inheritance of apomixis in buffelgrass (Taliaferro and Bashaw, 1966) and 2) a breeding scheme to produce true-breeding apomictic F1 hybrids that has resulted in the release of three buffelgrass cultivars (Higgins, Llano, and Nueces, Bashaw, 1968, 1980a).

Data from self pollination and from crosses of sexual buffelgrass (B-1S) with obligate apomictic buffelgrass and birdwoodgrass, suggested that MOR was conditioned by two genes with epistasis. When B-1S was self-pollinated, progeny segregated for MOR in a ratio of 13 sexual to 3 obligate apomictic plants. When B-1S was crossed with two different obligate apomictic buffelgrass genotypes, F1 hybrids were recovered in a ratio of 5 sexual to 3 obligate apomictic plants. The data from self- and cross-pollination fit ratios expected if MOR is controlled by two different genes with epistasis favoring the dominant expression of the gene for sexuality.

On the basis of these studies, it was postulated that sexual B-1S had the genomic formula AaBb and the two apomictic genotypes Aabb. The double recessive aabb would be expected to be sexual because of the absence of dominant gene A. Further confirmation regarding genetic control of

apomixis came from hybridization of B-1S with obligate apomictic *C. setigerus*. The F1 hybrids from this cross segregated in a 1:1 ratio of sexual to obligate apomictic plants, indicating that this *C. setigerus* accession represents the only other genotype for apomixis (AAbb) possible under this hypothesis.

We are unaware of any attempt to elucidate biochemical control of apomictic reproduction in plants, although the frequency of sexual embryo sacs has been observed to be sensitive to environment (Grazi et al, 1961; Gounaris et al, 1990; Gounaris et al., 1991; Knox, 1967; Quarin, 1986; Sherwood et al., 1980). Hypothetically, we might assume that dominant gene *A* activates somatic nucellar cells (normally senescent) and suppresses sexual development resulting in only the formation of functional unreduced (mitotic) embryo sacs. A second gene, *B*, when dominant, nullifies all action of gene *A* (epistasis) and restores sexuality. This model also provides a feasible explanation for facultative apomixis if we assume that incomplete action of gene *A* could result in failure to suppress the sexual mechanism allowing for both MOR to occur simultaneously in the same flower or plant. Sexual buffelgrass plants, believed to be homozygous (aabb) for MOR, have recently been identified and should provide additional germplasm for studying genetic regulation of apomixis in buffelgrass.

At this time, our research emphasizes methods to improve the frequency of gene transfer through the fertilization of unreduced aposporous eggs (2n+n or BIII hybridization). Winter- hardy pentaploid (2n+n) buffelgrass germplasm, collected by E.C. Bashaw and A.J. Oakes in South Africa in 1976, has provided a functional example of the benefits and problems associated with this phenomenon. Initial attempts to use pentaploid germplasm were motivated by the need to transfer winter-hardiness genes from pentaploid to tetraploid buffelgrass. From these preliminary crosses between B-1S x pentaploid buffelgrass, it was concluded that genes controlling winter-hardiness were located on the alien (x=9), non-buffelgrass genome, since only progeny which contained 45 chromosomes [18 (B-1S) + 27 (pentaploid)] were winter-winter-hardy. Because of the poor pollen quality of pentaploid buffelgrasses, few hybrids were recovered using sexual buffelgrass as the female parent and only a single plant contained all 45 chromosomes.

Subsequent efforts to transfer genes for winter-hardiness have attempted to exploit the low to moderate levels of sexuality observed in some pentaploid genotypes. Cytological investigation of several facultative apomicts (i.e. PI 409266, 409277, & 409704), indicated levels of potential sexuality as high as 14 percent (Hussey et al., 1991).

Using a chemical gametocide to aid the emasculation of the female parent, crosses were made between PI 409704 (*C. ciliaris*) x PI 193444 (*C. setigerus*). Birdwoodgrass (C. setigerus) was used as the male parent to facilitate the identification of hybrid progeny (Bray, 1978). All nine progeny (2N=63;2n+n) which were recovered from this cross originated from the fertilization of unreduced eggs and were highly fertile, true breeding obligate apomicts with characteristics of both parents (Bashaw and Hignight, 1990).

Based on these results, it was concluded that even though the frequency at which 2n+n hybrids were recovered is low (ca. 1%), fertilization of unreduced eggs represents a method which can be used for germplasm enhancement of apomictic species. While fertilization of unreduced eggs have been reported previously (Harlan and de Wet, 1963; Pepin and Funk, 1971; Dale et al., 1975), and 2n+n hybrids have been released as commercial cultivars, we are unaware of systematic attempts to use 2n+n hybridization as a method for germplasm enhancement and plant improvement.

Presently, our research focuses on the use of *P. flaccidum* Griseb., to study fertilization and gene transfer in *Cenchrus* and *Pennisetum*. *Pennisetum* flaccidum is a tetraploid facultative apomictic whose protogyndrous flowering behavior eliminates the need for emasculation. Hybridization experiments between *P. flaccidum* (PI 220606 and 315868) and P. mezianum Leeke (PI 214061) have allowed us to recover progeny derived from the fertilization of both reduced (n+n) and unreduced (2n+n) eggs (Bashaw et al., 1992).

Sexual B_{II} (n+n) hybrids (*P. flaccidum* x *P. mezianum*) are sterile due to high rates of male and female abortion. However, apomictic BII hybrids which reproduce through pseudogamy, are sterile when self-pollinated, yet fertile when pollinated with viable pollen (i.e. *P. mezianum*). In contrast all B_{III} (2n+n) hybrids, derived from the fertilization

of unreduced apomictic eggs, are highly fertile regardless of their MOR (sexual or apomictic). The ability to recover highly apomictic B_{III} hybrids provides an opportunity for transfer and expression of entire genomes and to synthesize new, fertile, true breeding 2n+n genotypes.

Field evaluations of four 2n+n interspecific hybrids (*P. flaccidum* x *P. mezianum*) during 1990-1991 were promising. These hybrids were superior to both parents in seed production, intermediate in forage production, yet they exhibited the good winter-hardiness of the female parent (*P. flaccidum*). Sexual, facultative, and obligate apomictic hybrids recovered from these experiments will provide an excellent model system for studying apomictic reproduction.

We believe that future research should include comprehensive investigations on methods for gene transfer among related sexual and apomictic species. Transfer of genes for method of reproduction using both conventional (n+n) and B_{III} hybridization has been demonstrated as a realistic approach to plant improvement. Molecular and biochemical research is urgently needed to understand gene action and provide a basis for transformation studies. Even the remotest possibilities for man to control and manipulate nature's method of cloning plants through seed deserve serious consideration.

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Physiology and Genetics of Apomixis in *Cenchrus*

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Strategies for Manipulating Apomixis

The goals of the apomixis CRIS project at the Pasture Lab are to elucidate the ontogeny of apospory and to clone a gene for apomixis. Research is progressing in both areas: we are 1) measuring the DNA levels of key cell types in developing apomictic and sexual embryo sacs, 2) developing a new model for apomixis inheritance in buffelgrass, *Cenchrus ciliaris* L. 3) preparing and characterizing subtraction cDNA libraries enriched in apomixis-associated sequences, and 4) initiating screening of buffelgrass lines for useful RAPD bands in preparation for an ongoing development of a genetic map for buffelgrass.

Physiological Regulation of Embryo Sac Type

All studies at the Pasture Labortory have involved plants derived from seed from sexual plant B-1s provided by Dr. E.C. Bashaw, USDA, TX. Embryo sac type is classified by viewing cleared pistils (Young et al. 1979). The Polygonum type of sac is considered to be meiotically reduced and sexual, while the Panicum type is considered unreduced and aposporous. Examination of 15-100 pistils per plant revealed the occurrence of both sac types in some plants, i.e. facultative apospory (Sherwood et al. 1980). Facultative apomixis occurs frequently in the material.

Many physiological treatments have been used in attempts to induce aposporous development in sexual plants or sexual development in aposporous plants (Gustine et al. 1989). DeGroote and Sherwood (1984) devised an in vitro system for delivering plant growth regulators (PGR) directly to involucres cultured aseptically on modified Murashige and Skoog medium. Cultures were established with involucres at the archesporial stage and were harvested after embryo sacs differentiated. The system was used to test effects of abscissic acid, gibberellic acid (GA), indole-3-acetic acid (IAA), and zeatin alone or in all possible combinations on growth and development of the ovules and embryo sacs of obligately sexual, obligately apomictic, and facultatively apomictic lines. The PGR neither suppressed production of reduced sacs nor induced apospory in a sexual line. They did not suppress apospory or permit sexual development in obligately apomictic cultivar Higgins. Treatments of facultative line 18-35 which included GA and omitted IAA, increased the ratio of reduced to unreduced sacs.

Gounaris and Sherwood (unpublished) screened about 50 compounds for potential to alter reproductive mode. These included PGRs, anti-PGRs, inhibitors of translation and transcription, and inhibitors of pathways of plant metabolism. Compounds were applied to roots, leaves, or inflorescences of premeiotic plants. None of the organic compounds tested changed reproductive type. Application of stressful amounts of 1 M ammonium sulfate solution to soil for 1-2 weeks before embryo sac formation induced multiple sexual embryo sacs in a line that normally produced only single sexual sacs (Gounaris et al. 1990). Further study of the effects of salt stress showed that the induction of multiple sacs in sexual lines was ion nonspecific (Gounaris et al. 1991). Salt stress induced formation of Polygonum type sacs in 4-13% of pistils of obligately aposporous lines. We postulated that salt stress suppressed developmental priority of nucellar embryo sacs over megaspores in aposporous lines, permitting completion of normal megagametophytes. We further considered that when multiple sexual sacs formed, each was derived from a separate megaspore of the linear tetrad, salt stress acting to abolish the normal developmental priority of the chalazal megaspore. These findings are the basis of research in progress. 1) We are using Feulgen staining of nuclear DNA to determine whether the nuclear DNA content of salt induced embryo sacs of aposporous plants is at the meiotically reduced or unreduced level. 2) We are examining megasporogenesis and megagametogenesis in salt stressed pistils. 3) We are examining selfed progeny of salt stressed obligate apomicts to seek offtype plants as evidence for induction of functional sexual sacs.

Inheritance of Embryo Sac Type

In the original model for inheritance of apomixis in buffelgrass (Crop Sci. 6:473, 1966) segregations upon selfing B-1s or crossing B-1s with apomicts were consistant with the hypothesis that two disomic genes (A and B) determined apomixis in buffelgrass. It was postulated that dominant allele A was required for apospory and dominant allele B was epistatic to A. We reexamined the inheritance of embryo sac type (Sherwood and Berg, in preparation). From progeny of selfed B-1s (putative genotype AaBa) we selected 5 obligately sexual plants and 4 obligate apomicts. Sexual plants were selfed and intercrossed. Each apomict was hybridized with several sexual plants. None of the sexual plants was indicated to have the putative epistatic

gene. Results were consistent with a model postulating that apospory is regulated by a single tetrasomic gene, dominant for apospory, with incomplete penetrance in some genetic backgrounds.

Strategies for cloning the apomixis gene from buffelgrass

Since 1986, we have been pursuing a number of strategies for obtaining cDNA clones associated with apomixis in buffelgrass: 1) isolate and characterize an apomixis-associated protein, and then obtain cDNA inserts based on the N-terminal partial sequence, 2) convert an apomictic to a sexual plant by mutagenesis via gene tagging, then clone the sequences adjoining the DNA tag, 3) differentially screen apomixis cDNA libraries with sexual and apomictic probes (DNA or antibodies), 4) prepare subtraction libraries containing only apomixis-associated sequences, and 5) prepare RFLP and/or RAPD maps to find markers linked to apomixis. We were not able to find an apomixis-associated protein; the numbers of plants required for screening tagged sequences was prohibitively high, so we did not initiate that project; and differential screening of expression libraries with antibodies to sexual and apomictic proteins was not successful. The most promising approaches are the subtraction library and RAPD strategies, which are now underway at the Pasture Lab.

Apomixis-associated cDNA Libraries

cDNA libraries were prepared with purified poly A^+ mRNA isolated from florets of Higgins (apomictic) or B-11-7 (sexual) at the stage of meiosis. cDNA was cloned into lambda gt23A following instructions of the Gibco-BRL kit (Hulce, Gustine, and Sherwood, unpublished). Sequences common to both plant types were subtracted out to produce cDNAs representative of apomixis-associated sequences. Two procedures were used: 1) restriction endonuclease (RE) method and 2) biotinylation/streptavidin (B/S) method. In the RE method, insert DNAs of sexual and apomictic cDNAs were removed with Not I and Sal I; single-stranded ends of sexual cDNA inserts were removed with S1-nuclease; and the insert cDNA (sexual) digested with Alu I and Rsa I (all fragments blunt-ended). One part of apomictic cDNA (not blunt-ended) was hybridized with a 50 to 100-fold excess of sexual cDNA fragments: sequences common to both reproductive types hybridized to produce double-stranded, blunt-ended, non-clonable sequences, while apomictic sequences only hybridized to each other and produced clonable hybrids, which were then cloned into lambda gt22A. In the B/S method, cDNA inserts from a sexual plant were labeled with biotin, combined with cDNA inserts from an apomictic plant, denatured and allowed to hybridize. Double-stranded cDNA containing sequences common to both plant types will always be labeled with biotin, whereas those dsDNAs having apomixis sequences will not be biotinylated. The mixture was mixed with streptavidin-coated beads and centrifuged; the supernatant contained only apomixis-associated sequences, which were cloned into lambda gt22A. The subtraction libraries were differentially screened with biotinylated poly A^+ RNA (Hulce, Gustine, and Sherwood, unpublished). So far no plaques have been identified in two of seven subtraction libraries that consistantly and preferentially hybridize to apomictic biotinylated poly A^+ RNA.

Buffelgrass Genetic Map

A cDNA library in lambda gt11 was prepared with purified poly A^+ mRNA isolated from florets of Higgins (apomictic) and B-11-7 (sexual) at the stage of meiosis Gounaris, Gustine, and Sherwood, unpublished). Lambda gt11 DNA containing cDNA insert was obtained from amplified, randomly selected plaques and biotinylated. These probes were used to detect RFLP bands on Southern blots of genomic DNA from apomictic and sexual buffelgrass. These probes give very weak signals, presumably because the lambda gt11 DNA dilutes the signal. We will amplfy the inserts by PCR to obtain useful probes. We are currently examining RAPD patterns produced from a combination of 11 oligonucleotide primers (10-mers) and five sexual and three apomictic buffelgrass lines. The RAPD procedure involves amplification of genomic DNA sequences using random oligonucleotide sequences as a primer. DNA bands amplified in this way are inherited in a Mendelian fashion.

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Apomixis in *Eragrostis*

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Brown and Emery (1958) were the first to suggest that apomixis occurs in the genus *Eragrostis*. They observed 4-nucleate embryo sacs that suggested the presence of diplospory in *E. curvula* and two closely related species. However, it remained for Streetman (1963) to describe in detail the occurrence of diplosporic apomixis in *E. curvula* and *E. lehmanniana*. In these early studies, germplasm described as *E. chloromelas* was probably E. *curvula* var. *conferta*. Descriptions of diplosporic apomixis by Streetman and other scientists suggest that, in at least some genotypes, five or six- nucleate embryo sacs including antipodals can develop in some ovules that are produced ameiotically. In contrast, Vorster and Libenberg (1984) suggest that only embryo sacs that develop meiotically have nuclei at the chalazal end of the developing embryo sac. Yet we know that the germplasm studied by Streetman (1963) was highly apomictic. Sexuality in *E. curvula* was not reported until 1971 (Voigt, 1971). Either the germplasm studied by Streetman had a different genetically determined developmental potential than that described in Africa by Vorster or some environmental condition caused the difference in behavior. Even if the difference has an environmental cause, it can not be assumed that environment caused a change from apomictic to sexual development when antipodals are produced. Despite ample opportunity, Streetman (1963) never observed the products of meiosis in any of the species described as apomictic, yet he observed it readily in sexually reproducing species.

Diploid sexual germplasm of *E. curvula* var. *conferta* was discovered in the late 1960s (Voigt, 1971). This was followed by the discovery of sexual tetraploid germplasm of the same grass and development of the first man-made *E. curvula* hybrids (Voigt and Bashaw, 1972). It was soon obvious that apomixis was facultative rather than obligate as originally thought (Voigt and Bashaw, 1976; Brix, 1974). Results suggested that hybrids could be either obligate sexuals or facultative apomicts, although some apomicts were highly apomictic, and in some cases may have been obligate apomicts (Voigt and Bashaw, 1976). When sexual plants were self pollinated, they produced variable progenies and the open-pollinated progenies of these S1 plants were also variable. Although this was not confirmed cytologically, I believe that, because the original parents were morphologically diverse, the conclusion that all of the S1 plants reproduced sexually is probably valid. If this conclusion is correct, and it certainly needs verification because progeny tests are notoriously unreliable when the level of apomixis is low (Voigt and Bashaw, 1976; Voigt and Burson, 1983), sexual plants do not contain all the genes necessary for expression of apomixis. In light of this evidence, the simplest genetic model would be that apomixis is dominant to sexuality and controlled by a single gene. This analysis assumes also that sexuality is the basic method of reproduction and that apomixis is derived from and may suppress but does not eliminate the potential for meiotic reduction. Thus, plants that demonstrate apomictic potential are classified as apomicts, i.e. facultative apomicts are considered apomicts because they have the genetic potential for ameiotic reproduction. The above analysis also assumes that additional genes control the level of apomictic reproduction in plants containing the dominant gene for apomixis.

Extensive progeny test data from numerous lovegrass hybrids indicate that when highly apomictic plants used as male parents are crossed with obligate sexual genotypes, about 50% of the hybrids are apomictic and 50% are sexual, especially when plants suspected of being facultative apomicts are combined with the more obligate apomictic hybrids (Voigt and Burson, 1983; Voigt, unpublished). The clearest exception sometimes occurs when higher polyploid germplasms are used as male parents in crosses with sexual tetraploid plants. When a septaploid plant was used as the male parent, 90% of the hybrids were apomictic (Voigt and Burson, 1983). This result has been interpreted as a dosage effect. Of course, some of the apomictic hybrids could also have resulted from fertilization of unreduced eggs. Additional evidence from other crosses is needed to determine the exact relationship between ploidy of the apomictic parent and the ratio of sexual to apomictic hybrids.

L. Neal Wright and two of his students also conducted limited work on apomixis in *E. curvula*. Stalker and Wright (1975) doubled the chromosome number of sexual-diploid boer lovegrass, *E. curvula* var. *conferta*. The induced tetraploids were sexual and self-sterile. They were planted in a field of 'Catalina' boer lovegrass and the resulting 84 plants were assumed to be hybrids. According to the authors, all 84 hybrids reproduced sexually. However, the methods by which hybrids were classified as to mode of reproduction and evidence that the offspring were indeed hybrids were not presented. Later, Bussey and Wright (1978) reported on six of the "hybrids." One plant was highly apomictic and similar in appearance to the male

parent and was regarded as a contaminant. Four plants were reported to be sexual. The final plant was a facultative apomict. Mode of reproduction of these six "hybrids" was determined primarily by progeny test methods.

The results of Stalker and Wright (1975) are in complete contrast to those of Voigt and Bashaw (1976) and Voigt and Burson (1983). There appear to be two possible explanations: 1. Induced sexual-tetraploid germplasm of boer lovegrass is genetically different, for genes controlling mode of reproduction, from naturally occurring sexual-tetraploid boer lovegrass germplasm, and/or 2. Errors were made in identification and/or classification of mode of reproduction of the Stalker and Wright hybrids. The results of Bussey and Wright (1978), that contradict those of Stalker and Wright (1975), support the second hypothesis.

Two approaches appear necessary to fully resolve the questions raised above. One is to repeat the work of Stalker and Wright. Unfortunately, the induced tetraploid germplasm used in that work was not preserved when illness forced Neal Wright to retire. Thus, it will be necessary to develop new induced- tetraploid germplasm before this approach can be tried. The second approach is to develop facultative-apomictic germplasm to use as the female parent in hybridization with highly apomictic germplasm. Limited attempts to use facultative-apomictic plants as female parents have not been very successful (Voigt, unpublished).

We have attempted new crosses between naturally occurring sexual-tetraploid plants of boer lovegrass and 'Ermelo' weeping lovegrass. The resulting hybrids will be carefully studied by progeny test and cytology to determine accurately the mode of reproduction of the hybrids. Male and female parents are sufficiently different that hybrids should be obvious, compared to any selfs that may be produced. Based on past experience, most hybrids should be either highly sexual or highly apomictic, but some should be intermediate.

Facultative apomictic hybrids, that are intermediate in the rate at which they reproduce by apomixis, will be selected to use as female parents in crosses with a different highly apomictic genotype, so that again hybrids can be distinguished from any selfs or from progeny produced apomictically. Mode of reproduction of these hybrids will be studied to determine if the ratio of apomictic to sexual hybrids differs from those obtained from crosses between sexual and apomictic plants. If the ratios differ, then we have correctly classified the mode of reproduction of our sexual germplasm. If the ratios are the same, then our sexual germplasm has not been classified correctly and despite its performance in progeny tests and in cytological studies, it is genetically apomictic and additional unknown genes have masked its true genotype. If that proves to be the case, then Stalker and Wright's findings are probably correct and apomixis would be recessive to sexuality, rather than dominant (Voigt and Bashaw, 1976).

Our finding that sexual hybrids when self pollinated do not give rise to apomictic offspring was based on progeny tests. Because of the potential inaccuracies of progeny tests, this work needs to be repeated using cytological analysis. This work, and the work described above, is needed to clarify the genetics of apomixis in a diplosporous apomict and to determine the inheritance of apomixis in the *E. curvula* complex. This work is also important because apomixis is much easier to study in *E. curvula* than it is in the diplosporous apomict *Tripsacum dactyloides*, and we may be able to use the *E. curvula* system of apomixis as a model system for T. dactyloides.

We have recently discovered sexual reproduction in a diploid form of lehmann lovegrass, *Eragrostis* lehmanniana, (Voigt et al., 1992). This species is related to the *E. curvula* complex, but its closeness to the complex is unclear. Sexual reproduction was previously unknown in this grass. In studying this germplasm we observed one ovule containing a greatly enlarged megaspore mother cell that would normally be considered indicative of an embryo sac developing apomictically. This is our first observation of this type in sexual-diploid germplasm. Of course we do not know if a developing embryo sac of this type could develop further in diploid germplasm. However, we do know that occasional unreduced eggs can occur in boer lovegrass, because a tetraploid plant was recovered from a diploid X tetraploid cross.

Probably, when unreduced embryo sacs are occasionally produced in diploid germplasm, they must be fertilized in order to function. However, because our cytological analysis of apomixis usually focuses on nonreduction (absence of meiosis or its products) rather than nonreduction and parthenogenesis, errors may be possible. That is,

especially in plants with a low level of nonreduction, we could overestimate the amount of apomixis, if nonreduction sometimes indicates an unreduced gamete that must be fertilized to produce a new individual. Perhaps even in tetraploids we should be cautious in concluding that a plant with a very low level of nonreduction is a facultative apomict, or in estimating the amount of apomixis in a facultative apomict when our observations are based only on the occurrence of nonreduction. It is possible that, at least in diplosporous apomicts, our cytological determination of mode of reproduction could overestimate the amount of apomixis.

An aspect of our apomixis research not discussed above are studies of the use of apomixis in practical plant breeding (apomictic breeding). Although much has been written concerning the potential of apomictic breeding, relatively little has actually been accomplished. The best examples are in the breeding of turf cultivars of *Poa pratensis*. Cultivars of *Cenchrus ciliaris* and *Panicum maximum* have been released, but their extent of adoption by producers is relatively small or unknown. Thus, a part of our apomixis research is to characterize variation released through apomictic breeding, to identify possible problems encountered during apomictic breeding and suggest possible solutions, and to develop useful new germplasm. The primary focus of this work has been forage quality and yield and winter hardiness. Manipulation of drought resistance in *Eragrostis* through apomictic breeding also should have great potential.

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Apomixis in *Paspalum*

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Paspalum is a large diverse genus that consists of more than 300 species. Apomixis is prevalent in the genus with the type being apospory. There are four reports of diplospory but all the plants were asynaptic or desynaptic (Chao, 1964, 1974, 1980). Published information about apomixis in this genus is limited primarily to the identification of apomictic species and the description of the apomictic mechanism (supplemental citations). An exception was a study of the inheritance of apomixis in bahiagrass, *P. notatum* (Burton and Forbes, 1960). The chromosomes of diploid, sexual Pensacola bahiagrass were doubled resulting in a sexual, induced autotetraploid that was cross compatible with natural apomictic tetraploids. These hybrids segregated for method of reproduction and provided the necessary information for an inheritance study. Unfortunately, cross compatible sexual and apomictic germplasm at the same ploidy level is not available in most *Paspalum* species. There are a variety of other reasons for the lack of progress in determining the inheritance of apomixis in other *Paspalum* species. Most sexual species are self-pollinated which necessitates tedious hand emasculation for successful hybridization. Floret size and morphology cause the emasculation techniques to be somewhat ineffective. Differences in ploidy level, meiotic irregularities, low pollen viability, and many other inherent factors also impact the successful hybridization of these species.

Common dallisgrass, *P. dilatatum*, is an important forage grass that reproduces by apomixis. It is a natural hybrid that provides an unique opportunity to identify and manipulate the genes controlling apomixis. Any progress in identifying the genetic control of apomixis will substantially contribute to the basic understanding of this reproductive phenomenon and will ultimately result in the improvement of this economically important species.

In 1958 Bashaw and coworkers determined the cytology and reproductive behavior of several dallisgrass biotypes (Bashaw and Forbes, 1958; Bashaw and Holt, 1958). They reported that common dallisgrass was an obligate apomict with 50 chromosomes which associated as 20 II and 10 I during meiosis. A yellow-anthered biotype from Uruguay was sexual and had 40 chromosomes which paired as 20 II. This biotype is the only source of sexual germplasm in the species. After numerous attempts, Bennett successfully crossed the yellow-anthered and common biotypes (Bennett et al., 1969). One sexual hybrid was recovered which had 45 chromosomes that associated as 20 II and 5 I. These findings were significant because they demonstrated that both biotypes have two similar genomes, I and J, and provided the first insight as to the location of the genes controlling apomixis in common dallisgrass. The univalents in the hybrid are five of the 10 univalents in the common biotype. Because the hybrid was sexual, it appears the genes for apomixis are absent; therefore, they must be located on one or more of the missing univalents. Hundreds of F_2 progeny were observed and all appeared sexual. By the F_3 generation, the univalents were absent in all plants examined. Cytologically and phenotypically the plants were similar to the yellow-anthered biotype.

These findings provided the impetus for initiating a phylogenetic investigation of the genus with the primary objectives: (1) to identify the progenitors of apomictic common dallisgrass; (2) to release the variability in this biotype; and (3) to reconstruct a highly fertile ecotype of common dallisgrass. Eventually the phylogenetic relationships among a number of species were elucidated and the genome formulas IIJJ and IIJJX were assigned to the yellow-anthered and common biotypes, respectively (Burson, 1983). Therefore, the genes controlling apomixis in common dallisgrass are located on one or more of the chromosomes of the X genome. Because the genes are in the haploid condition, this provides an unique opportunity to identify and manipulate the genes for apomixis.

After it was established that the yellow-anthered and common biotypes shared two genomes (I and J), the yellow-anthered biotype was used extensively as a cytological substitute for the apomictic common biotype. Consequently, initial research focused on establishing genome relationships. When crosses were between sexual species, essentially all hybrids produced were sterile. When the yellow-anthered biotype was crossed with apomictic polyploids, some of these interspecific hybrids had a limited propensity for apomictic development but functional apomictic sacs never developed (Burson, 1985).

Recently two new hexaploid dallisgrass biotypes, Uruguayan and Uruguaiana, were obtained during a plant collection trip in South America. Both are apomictic and have 60 chromosomes (Burson et al., 1991). This new germplasm has played an integral role in the progress of the phylogenetic investigation and provided a better understanding of

apomixis in dallisgrass. Without this germplasm, the program would presently be at a stalemate. The Uruguayan biotype has contributed significantly to our understanding of apomixis in dallisgrass as well as the origin of common dallisgrass. It also may be the key in developing superior dallisgrass germplasm because when crossed with the sexual yellow-anthered biotype, the heterozygosity locked in this apomictic biotype is released in the hybrids.

(1) The Uruguayan biotype, genome formula IIJJXX, was crossed with the yellow-anthered biotype. More than 40 hybrids were produced. Some were cytologically and phenotypically similar to the common biotype. It was proposed that common dallisgrass originated from a natural cross between these two biotypes or closely related types (Burson, 1991a). Progeny tests show that some of the hybrids reproduce by apomixis and others are sexual. A myriad of types were expressed in the F_2 progeny of the sexual hybrids. This was the first time the heterozygosity locked in an apomictic dallisgrass biotype was released by crossing sexual and apomictic types. This unprecedented variation may be the initial step in obtaining an understanding of the control of apomixis in this species and provide the necessary germplasm for the eventual improvement of the species.

(2) The Uruguaiana biotype ($IIJJXX_2$) was also crossed with yellow-anthered dallisgrass and 5 hybrids were recovered. Cytological analysis revealed that all hybrids were facultative apomicts because sexual and apomictic sacs were produced. However, progeny test data indicated that some hybrids were completely apomictic while others were partially sexual. The variability expressed in the F_2 progeny was similar to that observed in the F_2 progeny of the yellow-anthered x Uruguayan hybrids. It appeared these hybrids were facultative apomicts but the sexual sacs were not functional in some hybrids (Burson, unpublished data).

(3) Vaseygrass, *P. urvillei*, a close sexual, tetraploid relative of dallisgrass which has the genome formula IIJJ, was crossed with the Uruguayan and Uruguaiana biotypes. Their hybrids behaved similarly to some of the yellow-anthered x Uruguaiana hybrids previously mentioned. Cytologically both sexual and apomictic sacs were present in the ovules of all hybrids but their F_2 progeny were extremely uniform. This indicated these hybrids were facultative apomicts but only the apomictic sacs were functional. Two off-type plants were found in the progeny of a vaseygrass x Uruguayan hybrid. They were B_{III} hybrids that originated from the fertilization of an unreduced egg in the F_1 hybrid. Their chromosome number (2n=7x=70) and appearance indicated that vaseygrass was the male parent. Cytologically the B_{III} hybrids also were facultative apomicts (Burson, unpublished data).

(4) Recently the sexual, 45-chromosome yellow-anthered x common dallisgrass F_1 hybrid (Bennett et al., 1969) was crossed with the Uruguayan biotype. Two trihybrids were recovered; one had 52 chromosomes which associated as 22 II and 8 I and the other had 53 chromosomes which associated as 23 II and 7 I. Examination of the embryo sac revealed a tendency for both sexual and apomictic development. However, by anthesis most of the apomictic sacs aborted and 58% of the mature ovules contained a sexual sac (Burson, 1991b). Variability among the progeny of the trihybrids confirmed the sexuality. These findings indicated that members of the X genome in the Uruguayan biotype were similar to those of the X genome in common dallisgrass. Thus, it is feasible to use the meiotically stable Uruguayan biotype as a substitute for common dallisgrass in studying apomixis. Because the two trihybrids are partially sexual, they can be crossed with either the common or Uruguayan biotypes to develop an aneuploid series for the chromosomes of the X genome. This germplasm could be extremely important in determining and identifying which chromosomes possess the genes for apomixis with the use of molecular approaches such as molecular genetic markers.

In this genus, as in most apomictic species, the diploids are sexual and apomixis occurs at higher ploidy levels. Sexual tetraploid germplasm is usually obtained by doubling the chromosomes of a sexual diploid with colchicine. Quarin and Hanna (1980) doubled the chromosomes of sexual, diploid *P. hexastachyum* (2n=2x=12) and the induced tetraploids and hexaploids were facultative apomicts. Recently a sexual diploid form of brownseed paspalum, *P. plicatulum*, was treated with colchicine and the progeny from the induced tetraploids were uniform indicating they were apomictic (Burson, unpublished data). Addition diploid seed have been treated with colchicine in an attempt to confirm this result. These findings are an exception to the norm and place a different perspective on the genetic control of apomixis in these grasses. This contradicts the theory

that apomixis is controlled by a single gene in these two species.

The fertilization of unreduced eggs in apomictic embryo sacs occurs more frequently and is more important than was previously thought. Since using the hexaploid dallisgrass biotypes in the hybridization program, more B_{III} hybrids have been recovered in the last 5 years than during the past 20 years. This phenomenon is not well understood but it provides an unique means for creating new apomictic types. If the frequency of this process is increased, it could be a valuable tool in breeding apomictic species.

In summary, it appears that the genes controlling apomixis in the common, Uruguayan, and Uruguaiana dallisgrass biotypes are located on one or more of the chromosomes of the X genome. However, there is a difference in the expression of the gene(s) for apomixis. In common dallisgrass when the genes are in the haploid condition, obligate apomixis is expressed, but in the F_1 hybrids between yellow-anthered and the 60-chromosome biotypes, with a similar haploid condition for the X genome, facultative apomixis is expressed. More conclusive information is needed before a model of the genetic control of apomixis in dallisgrass can be proposed. However, the most likely hypothesis to explain these results is that one dominant gene may control apomixis but additional regulating genes may determine the expression of facultative apomixis.

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Manipulating Apomixis in *Paspalum*

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The *Paspalum* genus contains more than 400 species, most of which are native to the Western Hemisphere. Many of the 50 species studied reproduce by apomixis (Quarin & Hanna, 1980).

Chromosome behavior, mode of reproduction and fertility characteristics were studied in diploid (2n = 12) and colchicine-induced tetraploid and hexaploid *Paspalum hexastachyum* Parodi (Quarin & Hanna, 1980). Diploids were sexual but induced tetraploids and hexaploids reproduced by facultative apomixis. Diploids were self-incompatible but cross-compatible as were diploid Pensacola bahiagrass, *Paspalum notatum* var. saure Parodi plants (Burton, 1955). The plants were self-compatible at the tetraploid chromosome level.

Breeding Dallisgrass

The first report of apomixis in *Paspalum* involved *P. urvillei* Steud. (2n = 40) (U) *P. dilatatum* Trin (2n = 40) (M) and *P. dilatatum* (2n = 50) (D) (Burton, 1943). Five chance male sterile U x M hybrids found in 5000 spaced U plants pollinated with U and D pollen produced 57 (U x M) x U (2n = 60) hybrids, 100 (U x M) x D (2n = 60) hybrids and several hundred plants like their U x M parent (Burton, 1943). Uniformity of the parents, hybrids and their progenies indicated apomixis as their mode of reproduction. The hybrids showed greater flooding, drought and heat tolerance and when cut only once, yielded twice as much as U, M or D. When growing with U, M. and D, they set 0.39 to 3.14 percent caryopses by weight, too little for commercial propagation. U, M. D and their hybrids were bunch grasses.

Smith (1948) and Bashaw and Holt (1958) observed embryo sac development in common dallisgrass (CD) and concluded that it developed by obligate apomixis, apospory. A 25-year study of over 100 ecotypes noted marked uniformity with several slightly different mutants that bred true (Burton, 1962). Except for the U x M x D hybrids extensive intraspecific hybridization efforts gave rise to maternal offspring.

The best radiation seed treatment (20 hours of thermal neutrons) increased the R_1 mutants in apomictic prostrate dallisgrass *P. dilatatum* var. pauciciliatum 4-fold (Burton & Jackson, 1962). Radiation mutants bred true. Radiation did not induce sexuality in this obligate apomict and failed to increase its resistance to ergot, *Claviceps paspali* F.L. Stevens and J.G. Hall.

Breeding Common Tetraploid Bahiagrass

Common bahiagrass *Paspalum notatum* (2n = 40) is a warm season South American species. It is a slow spreading (30 to 45 cm/yr) sod-forming bunchgrass. It is propagated by seed borne on culms usually less than 50 cm tall and it flowers from June to October. It has red stigmas. It is a popular pasture and lawn grass in Florida and the lower quarter of the Gulf states.

Objectives of the genetic improvement of common bahiagrass (CT) since 1936 have included increased forage and seed yield, improved cold and drought tolerance and ergot resistance in an apomictic plant.

When for several early years progenies of bahiagrass introductions were uniform, selections lost no vigor when selfed and hybrids were identical with their female parent, apomixis as the mode of reproduction was suspected. Confirmation of this opinion was made possible when a fertile white stigma (WSCT) plant, a male sterile (MSWSCT) plant and the sexual red stigma Pensacola bahiagrass diploid (Pd) were found. Then MSWSCT x red stigma (RS) CT plants gave several RSCT (2n = 60) hybrids and many MSWSCT progeny (Burton, 1948). MSWSCT x Pd plants gave several RS hybrids (2n = 50) and many MSWSCT progeny. Pd x CT plants gave several RS hybrids (2n = 30).

I. Collecting and Testing Ecotypes

Beginning in 1936 and continuing for more than 30 years, some 80 ecotypes of bahiagrass from Brazil, Uruguay, and northern Argentina were collected and studied.

Most of the 80 ecotypes evaluated were typical, low-growing broadleafed common types that failed to survive a severe winter at Tifton. All of the common types were obligate apomicts. No sexual plants were found among them. The chromosome number of those examined was (2n = 40).

Argentine and Paraguay 22, formerly PI 148996 and PI 158822, respectively, were the most winterhardy and the most promising of the apomictic tetraploids. Compared with common in a 3-year clipping test, Argentine produced twice as much dry matter and protein as common. Paraguay 22 was more drought tolerant than Argentine and much more

drought and cold tolerant than common. Argentine bahiagrass is used for lawns in Florida and both grasses are grown for pastures.

II. Creating Apomictic Hybrids

Our failure to find sexual plants in the tetraploid ecotypes caused us to create 10 sexual tetraploids by doubling the chromosomes of the sexual Pensacola bahiagrass (2n = 20) with colchicine (Forbes & Burton, 1957). In 1956, three induced sexual tetraploids, (PT2), (PT4), and (PT10), were hybridized with two obligate apomicts, (MH) from Monzon Heber, Uruguay and (WS), a white stigma common bahiagrass mutant. Four hundred forty F_1 hybrids were produced. The occurrence of apomictic F_1s in each of the four crosses PT2 x WS, PT2 x MH, PT4 x MH and PT10 x MH indicated that both WS and MH carried dominant factors controlling apomixis. The occurrence of apomictic plants in the progenies of the sexual plants from these crosses indicated that WS and MH also carried recessive factors for apomixis (Burton & Forbes 1960, Forbes & Burton 1961). Two of the three PT x MH hybrids gave apomictic (A): sexual (S) F_1 ratios of 1:2.5 and 1:3.8. F_2 progeny of their sexual F_1s gave an A:S ratio of 1:39.8 and 1:30.4. Assume apomixis in these crosses was controlled by either a dominant factor D or an independent recessive factor a. Then sexual [PT2, PT4 or PT10 (dddd x AAAA) x MH (Dddd aaaa)] would have given A:S ratios of 1:2.5 in the F_1. Sexual F_1s (*dddd AAaa*) would have given tetraploid F_2 A:S ratios of 1:35. The observed F_1 A:S ratio 1:25 and the F_2 A:S ratio of 1:33.6 of PT2 x WS indicates that the WS tetraploid had a much lower frequency of the D factor than MH but like it was homozygous for the dominant A factor.

In a 1963 three-year 81-entry small plot test, 36 apomictic F_1 hybrids yielded 26% more than their 42 apomictic F_2s. The top entry, PT10 x MH 54, after topping a 5-year small plot test was named (Tifton 54). In later tests of more apomictic F_1s from sexual WSBs x 26 apomictic introductions, none were better than Tifton 54 in forage yield, seed set, seed yield, and IVDMD.

III. One Facultative Apomict

A semi-dwarf tetraploid from Brazil (B) interplanted with diploid Pensacola bahiagrass (Pd) 17-29 produced one triploid in a progeny of several hundred maternal plants (Burton & Hanna 1986, Hanna & Burton 1986). When pollinated with Pd pollen, the triploid produced 1 sexual diploid, 142 maternal triploids and 1 fertile tetraploid. The tetraploid produced 35 selfed and 70 open-pollinated (2n = 40) plants. Open-pollinated seed harvested from these plants in 1982 produced uniform progenies whereas seed harvested from them in 1983 gave variable progenies. Cytological studies and progeny tests indicated that the semi-dwarf tetraploid (B) was a facultative apomict. This was confirmed by controlled crosses.

IV. Environmental Stress to Break Obligate Apomixis.

Seed harvested from obligate apomicts from midsummer to frost at 109 m and 584 m elevations for three years failed to break apomixis (Burton 1982a). Stressing obligate apomicts by withholding water and nutrients during seed production also failed to make them sexual.

V. Mutation Breeding to Improve Seed Production of Tifton 54

A study of more than 7000 spaced seedling plants of Tifton 54 from 13 ethyl methane sulfonate (EMS) in water soaking treatments failed to reveal distinctly different M_1 mutants. Plants receiving 16 g kg^{-1} EMS were shorter than those receiving the much lower dosage treatments but no distinctly novel morphological characters were observed.

Inflorescences harvested from 100 plants selected at random within each treatment block had very low seed set. Although the highest treatments of 12 and 16 g kg^{-1} EMS soaked for 16 hours increased average seed set slightly, further testing showed no significant improvement in seed set as a result of EMS seed treatments.

VI. Efforts to Create Apomictic Diploids

Martin Wilson in Dr. I. Vasil's laboratory, Univ. of Florida, obtained diploid and tetraploid callus with roots from cultured anthers of three apomictic tetraploids but was unable to get shoots (unpublished).

Another culture of six apomictic tetraploid genotypes failed to develop signs of callus formation, embryoid development

or multicellular pollen formation (Luvick & Ozias-Akins, unpublished).

Crossing self-incompatible diploid Pensacola bahiagrass 17-29 with six apomictic tetraploids produced four apomictic triploids and 1370 diploids that resembled 17-29. Apparently, 17-29 set seed by the mentor effect and produced a selfed progeny of 1370 plants (Nettencourt, 1977).

VII. Recurrent Restricted Phenotypic Selection Population Improvement.

1974 Base Population

A 1000 plant base population was established in 1974 from an isolated increase of 176 of the best sexual tetraploid plants in 637 F_1 hybrids between five white stigma sexual plants and three red stigma apomictic male plants. These eight plants traced their origin to the F_2 generation of the PT2 x WSB cross where evidence suggested that apomixis was controlled by a single recessive factor. This population was advanced one cycle per year through cycle 4 by visually selecting the five best plants in 25 plant blocks in 1000 spaced plant populations and intermating three culms from each in the laboratory. This recurrent restricted phenotypic selection (RRPS) method was used successfully to increase yields of Pensacola bahiagrass (Burton, 1982b).

The January 3-4, 1979 temperatures of -10 C killed many of the plants in the old field plantings of cycles 3 and 4. The survivors of cycles 3 and 4 were intermated and advanced in the usual way to cycle 5 spaced planted in the field in 1981.

In October 1982, fresh plant yields of the spaced plants in cycles 4 and 5, growing side-by-side ranged from 0 to more than 2200 g per plant. Frequency tables of the plant yields in each cycle showed cycle 5 with a higher percent of the high-yielding plants than cycle 4. Open-pollinated progenies of the better plants in cycle 5 revealed that most of them were sexual and work with this population was discontinued.

1983 Base Populations

The 1983 sexual female (SF) population was produced by intermating 23 F1 hybrids between four sexual white stigma female plants and six red stigma apomictic plants. The white stigma plants and one of the apomict RSB2 were the best in cycle 5 of the 1974 base population where apomixis was controlled by the recessive a factor. The sexual female apomictic male (SFAM) population intermated the SF 23 population with an equal number of culms from six red stigma apomicts RS32, Tifton 54, introduction PI 158822 from Paraguay, MH from Uruguay and I1 and I6 from Brazil. All but RSB2 probably carried both dominant and recessive factors that could control apomixis.

The SF and SFAM population were advanced through three RRPS generations. In 1987, the 6 plants of the 165 entries of each population were planted in 3- plant plots to permit the uniformity test for apomixis. Fresh weights of each plant were taken. Only 9 of the top yielding SF entries were apomictic, whereas 19 of the top SFAM entries were apomictic. All of the 9 SF apomictics came from apomictic females whereas all of the 19 SFAM entries had sexual female parents and probably dominant apomictic males.

In a replicated 3-year small plot test seeded May 4, 1989, the best apomictic from the SFAM population, #7, yielded significantly more dry matter than the Argentine bahiagrass check. It also surpassed Argentine bahiagrass in ergot resistance and percent seed set.

Conclusions

Apomixis in tetraploid bahiagrass is controlled by independent dominant and recessive factors. Until a sexual tetraploid can be found or created, it and similar *Paspalum* species can best be improved by collecting and testing apomictic species. This procedure may produce a better genotype, find the needed sexual plant and provide an excellent gene bank when apomixis is broken.

To date, anther culture and hybridization have failed to create apomictic diploids. Without apomictic diploids, tetraploid inheritance that produces only one homozygous recessive in 36 F_2s and loss of vigor (26%) from the F_1 to the F_2 makes use of recessive factors to create superior apomictic cultivars difficult. Where the recessive factors are used, recurrent restricted phenotypic selection (RRPS) will be the most efficient method to create superior cultivars but the frequency of apomicts will be low.

Dominant factor apomicts add a static component to the RRPS population. They do not respond to the intermating

process and reduce the number of plants that do. However, this study indicates that dominant factors can create high-yielding apomicts. We will search for an apomict with excellent seed pro- duction and combining ability that, like MH, can create F1s with apomictic to sexual ratio of at least 1 to 2.5. RRPS will be applied to a completely sexual white-stigma population to improve its performance and create a few out- standing plants to be mated to our superior apomict. Red stigma F1s will be screened for apomicts that will be evaluated in replicated small plot tests.

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Apomixis in *Poa*

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Poa is a diverse genus of cool-season grasses containing approximately 300 species. Collectively known as bluegrasses in North America and as meadow grasses in Europe, *Poa* contains a wide variety of breeding systems including apomixis and dioecy (Table 1). The representative species of *Poa*, both agronomically and botanically, is *Poa pratensis* L. also known as Kentucky bluegrass in North America and as Smooth meadow-grass in Europe. This chapter will be primarily focused on this species. Kentucky bluegrass is an important agronomic crop which serves simutaneously as nutritous forage, as unexcelled turf, and for conservation purposes protecting against soil erosion throughout the temperate climates world-wide.

The breeding system of Kentucky bluegrass was first described by Muntzing (1933) to be facultative apomictic. Later, investigations demonstrated that pollen fertilization of a somatic aposporous embryo sac egg was normally avoided, but that fertilization of polar nuclei was necessary for proper endosperm development and subsequent seed germination (Akerberg, 1939, 1943; Tinney, 1940). Today, the particular form of apomixis found in Kentucky bluegrass is termed pseudogamous apospory.

To varying degrees, Kentucky bluegrass is also capable of reproducing sexually and thus, is referred to as a facultative apomict. However, not all maternally-deviating, i.e. aberrant, progeny plants are the result of a sexual process. Unreduced (2n) aposporous embryo sacs initiate independently of the germ cell line, from somatic tissue, usually of the nucellar region. Meiotically derived embryo sacs, containing a reduced (n) egg, may coexist alongside one or more unreduced aposporysacs within the same ovary. Typically, during the time between egg mother cell reductional division and anthesis, the reduced meiotic egg dies or is outcompeted by an unreduced apospory embryo sac or by a rapidly developing apomictic proembryo. On occassion, a reduced egg survives to become fertilized by a pollen nucleus, developing into a sexually derived hybrid (n + n) plant. Alternatively, a reduced egg may also develop pseudogamously, without fertilization, into a polyhaploid (n) plant. In the event that pollen fertilization of an unreduced apospory egg is succusseful, the result is a triploid (2n + n) plant, referred to as a BIII hybrid. Thus, aberrant progeny plants, which do not resemble the maternal parent, have many different genetic origins. The variable factors involved in genetic origin are ploidy levels (haploids, diploids, triploids, and tetraploids), and self vs cross fertilization. In addition, there is also the possibility of irregular meiosis/mitosis leading to the production of fertilized or non-fertilized aneuploids (Grazi, Umaerus, and Akerberg, 1961). Therefore, the apomictic breeding system of Kentucky bluegrass is capable of generating and perpetuating extreme chromosomal variation. As a result of these different genetic origins, aberrant seed progeny should be examined cytologically for inclusion in research and breeding programs.

Apomixis research in Kentucky bluegrass has primarily utilized traditional embryology, cytogenetic, and segregational methods of analysis (van Dijk, 1991). Although these techniques have been rigorously applied, and in some cases improved (Young, Sherwood, and Bashaw, 1979), there are several inherent reasons why they are technically difficult to perform in Kentucky bluegrass. First, the aposporous embryo sac structure (eight-nucleate) closely mimics that of meiotically derived sacs, making it difficult determine embryo sac origin. In these cases, it is necessary to characterize the location of the embryo sac within the ovary, i.e. meiotic sacs are more centrally-located near the micropyle. Second, classical cytogenetic analysis is made difficult due to the complex series of polyploidy and aneuploidy within Kentucky bluegrass; chromosome numbers range continueously from 24 to 124 (Love and Love, 1975). Within this distributional range, modal peaks in the 49-56, 63-70, and 84-91 chromosome-number classes have been observed (Nielsen, 1946). However, chromosome numbers have been found to vary from ± 3 to as much as ± 30 chromosomes among somatic cells within a single genotype (McDonnell and Conger, 1984; Wu and Jampates, 1986) and from one sexual generation to the next (Clausen, 1961). Third, segregation of hybrids typically exhibit transgressive segregation (Bashaw and Funk, 1987), making the task of interpreting quantitative genetic data more complex.

Apomixis is an ideal breeding system once a superior plant has been identified. Apomixis, however, limits a breeder's ability to effectively perform hybrizations because it masks or inhibits the sexual process. As a result, ecotype breeding was the only breeding method, prior to 1970, that had produced commercially successful cultivars (Bashaw and Funk, 1987). Selecting naturally occurring ecotypes continues to be the most efficient method of cultivar development. However, many of the most successful ecotypes have wide distributions and are repeatedly collected from different

geographical locations (Duyvendak and Luesink, 1979). The difficulty in collecting unique types from the wild is placing more emphasis on using hybridization as a means of producing genetic variation.

Hybridization in a facultative apomitic system is possible only to the extent of the presence of sexuality and/or the breakdown of apomixis. Large parental differences exist in the frequency and the performance of aberrant progeny. The frequency of aberrant plants has been found to depend on the female parent, while the performance of hybrids depends on both the male and female parents (Hintzen, 1979). Because the frequency of aberrants is inherently low among highly apomictic parents, large numbers must be examined to identify progeny resulting from sexual hybridization. The most efficient means of screening such large numbers of plants is through space-planted progeny test nurseries (Tinney and Aamodt, 1940).

Many of the selections from intraspecific hybridizations are often found to be B_{III} hybrids (polytriploid), resulting from the fertilization of unreduced eggs (Pepin and Funk, 1971; Funk et al., 1973; Hintzen, 1979). The increased number of chromosomes, due to B_{III} hybridization, raises a concern among breeders as to the optimal number of chromosomes for best varietal performance. Pepin and Funk (1974) suggest that 2n=100 may be the upper limit of that optimum; while currently, some breeders consider 2n=100 to be too high (Funk, 1992, pers. comm.).

As many as ten subspecies of *Poa pratensis* have been recognized by different botanists (Hitchcock, 1950; Hubbard, 1984; Tsvelev, 1976). The most common subspecies are *P. P. pratensis* which is the typic subspecies, *P. p. angustifolia* which has narrow leaves 1-2 mm wide and is found on limestone soils, and *P. p. subcaerulea* Sm. which is more rhizomatous and grows in moist to marshy soils. To date, no information is available concerning the range of chromosome numbers or the hybridization potential among these subspecies.

The presence of polyembryonic seed and the production of identical and non-identical twin, triplet, and quadruplet seedlings, has been a useful tool for apomixis research in Kentucky bluegrass (Andersen, 1927; Akerberg, 1939; Nielsen, 1946; Duich and Musser, 1959). On two occasions, subhaploid-plants (2n=18 or 16) of Kentucky bluegrass were found to resemble *Poa trivialis* (Kiellander, 1942; Akerberg and Bingefors, 1953), indicating that *P. trivialis* may be one of the component progenitor-species of *P. pratensis*. However, not all subhaploids (2n=14) resemble *P. trivialis* (Neilson, 1945). Analysis of chloroplast-DNA by Soreng (1990) suggests that *P. compressa* may be a result of hybridization between *P. pratensis* and an unspecified diploid species with compressed culms. Further hybridization between *P. pratensis* and *P. compressa* continues to be a breeding tool (Dale, et al., 1975).

In nature, interspecific hybridization among *Poa* species results in introgression to such an extent that species classification is often difficult (Stebbins, 1950). The retention of pollen recognition systems within *Poa* species, combined with the asexual nature of apomixis, present many possibilities for apomictic research and breeding applications through interspecific hybridization (Clausen, Hiesey, and Nobs, 1962). Muntzing (1940) found that interspecific hybridization often resulted in a breakdown of apomixis resulting in partially or completely sexual F_1 hybrids. This lead to his hypothesis that the genetics of apomixis in Kentucky bluegrass was controlled by a delicate balance capable of being disrupted by the slightest genomic shock. For breeding purposes, apomixis may be regained from interspecific crosses by screening for aposporous recombinates in succeeding F_2 and F_3 generations (Akerberg and Bingefors, 1953). Examples of interspecific hybridization include *P. pratensis* X *P. compressa* (Dale et al., 1975), *P. pratensis* X *P. alpina* (Akerberg, 1942; Akerberg and Bingefors, 1953), *P. ampla* X *P. pratensis* and P. scabrella X *P. pratensis* (Hiesey and Nobs, 1982), *P. longifolia* X *P. pratensis* (Almgard, 1966; Williamson and Watson, 1981; Van Dijk and Winklehorst, 1982), and *P. arachnifera* X *P. pratensis* (refered to in Oliver, 1910).

An important contribution towards breeding *Poa* would be a method of manipulating the apomictic breeding system. Manipulation of apomixis in Kentucky bluegrass with environmental conditions has had limited success. Hovin et al. (1976) observed a slightly greater frequency of aberrants among field grown plants in locations promoting a longer flowering period, but found that cultivar uniformity was not seriously impaired. Grazi, Umaerus, and Akerberg, (1961) and Han (1969) observed a greater tendency towards sexuality under greenhouse conditions than in the field. Funk and Han (1967) and Hintzen and van Wijk (1985)

describe an effective environmental treatment for hybridization, which essentially, is to allow plants to flower early in the spring under greenhouse conditions with long daylengths using artificial lighting, and to pollinate either mechanically or by hand as early as possible after the stigmas emerge and are receptive. Pollinating at the time that the flower opens has been suggested to increase chances of fertilization (Bashaw and Funk, 1987) because the apomictic proembryo often begins development at or slightly before anthesis (Akerberg and Bingefors, 1953). Several methods of mechanical pollination have been described to facilitate hybridization (Hintzen and van Wijk, 1985; Riordan, et al., 1988).

A deeper understanding of the underlying genetic control would be a vaulable asset towards the manipulation of apomixis. However, many researchers, from past and present experience, have suggested that the underlying genetic system of *P. pratensis* may be too complicated to solve or understand (Muntzing, 1940; Grazi, Umaerus, and Akerberg, 1961; Bashaw and Funk, 1987). Muntzing (1940), studing F_2 and F_3 progenies of a sexual *P. pratensis* plant and of haploid and triploid plants, concluded that apomictic seed formation is recessive to sexual seed formation. Pepin and Funk (1974) suggested apomixis to be under mostly dominant control from an F_1 family between two highly apomictic varieties segragating for sexual and apomixis. Contradictory results from Akerberg and Bingefors (1953) were interpreted by Almgard (1966, p.54) as: "Evidently apomixis as a functioning system can here (in *Poa*) be described as completely recessive, while its basic prerequisite, the presence in this case of an aposporous embryosac, rather showed a dominant pattern of inheritance in F_1".

Table 1. Agronomically important *Poa* species and some select characteristics.

Common name	Scientific name	Chromosome number	Reproduction
Kentucky bluegrass	*P. pratensis* L.	(18) 24-124 (154)	apospory apomixis
Canada bluegrass	*P. compressa* L.	35-56	apospory apomixis
Fowl bluegrass	*P. palustris* L.	14,28,42,63	diplospory apomixis
Bulbous bluegrass	*P. bulbosa* L.	21,28,35,42	bulbiferous apospory
Rough bluegrass	*P. trivialis* L.	14	self-incompatable outcrossing
Annual bluegrass	*P. annua* L.	28	self-compatable gynomonecy
Big bluegrass	*P. ampla* Merr.	62-100	apospory apomixis
Texas bluegrass	*P. arachnifera* Torr.	56,84	dioecious
Mutton bluegrass	*P. fendleriana* (Steud.) Vasey	56	incompletely dioecious
Canby bluegrass	*P. canbyi* (Scrbn.) Piper	72-105	apomixis

In the future, the genetic mechanism of apomixis might best be investigated by examining the genetic origin of segregating progeny rather than from the overall frequency of aberrants, which is the whole of the apomictic process. This research effort will benefit from the application of two relatively new and rapid molecular techniques called flow cytometry (Michaelson, et al., 1991) and randomly amplified polymorphic DNA (RAPD) markers which utilize the polymerase chain reaction (PCR) (Williams, et al., 1990; Welsh and McClelland, 1990, 1991; Caetano-Anolles, Bassam, and Gresshoff, 1991). Application of these techniques to Kentucky bluegrass have demonstrated 1) that flow cytometry is capable of accurately distinguishing between haploid, diploid, and triploid (B_{III}) progenies, and 2) that RAPD markers are capable of distinguishing progeny plants resulting from cross-fertilization, and are useful for quantifying the accumulation of genetic material in B_{III} hybrids and the deletion of genetic material from polyhaploids (Huff, in prep.). When used in combination, flow cytometry and RAPD markers are powerful tools for dissecting the genetic origins of Kentucky bluegrass individuals.

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Apomixis in the Triticeae

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Apomixis has been reported in only one species of the Triticeae, *Elymus rectisetus* (Nees in Lehm.) A. Love and Connor, which is endemic to Australia and New Zealand and is known in these countries as Australian wheatgrass. *Elymus rectisetus* is a facultative diplospore.

Present Knowledge

Numerous publications discuss the natural distribution and forage characteristics of *E. rectisetus*. However knowledge of apomixis in this species is based on only three studies (Hair 1956, Crane and Carman 1987, Carman et al. 1991). Decisively little is known concerning the physiological or genetic control of apomixis in this species. A modest attempt to alter pollen formation and facultative expression of apomixis in several accessions of *E. rectisetus* with temperature treatments failed to yield conclusive findings (Crane and Carman, unpublished). Pollen fertility is generally low in *E. rectisetus* and some accessions consistently produce 2n pollen. Seed set is also generally low, though this trait, as well as pollen fertility, varies greatly among accessions and is strongly influenced by growing conditions.

Taxonomy, genome analyses and wide hybridization. *Elymus rectisetus* (2n=6x=42) is generally a weak perennial bunchgrass, though long-lived accessions do exist. In Australia, it occurs in the valleys and on the slopes of the Dividing Range from northeastern New South Wales to southwestern Victoria, in eastern Tasmania, and in the Lofty Mountains of South Australia. It also occurs infrequently in West Australia and in the Flinders Ranges of South Australia. *Elymus rectisetus* occurs sympatrically throughout much of its native Australian range with *Elymus scabrus* (R. Br.) A. Love and with *E. scabrus* var. *plurinervis* (formerly *Agropyron scabrum* var. *plurinervis* (Vickery 1950), both of which are hexaploids (2n=6x=42). Until recently these three taxa were grouped together as *Agropyron scabrum* (R. Br.) Beauv. The most recent official taxonomic treatment of this group (Love and Connor 1982) is in need of revision. Some of the needed revisions are (a) the changing of *E. scabrus* to E. scaber, which in Latin is the grammatically correct choice between scaber, scabra and scabrum (Connor 1991, personal communication), (b) the official synonimization of the Australian apomicts with the New Zealand *E. rectisetus* apomicts, which probably originated from Australia during historical times (Love and Connor 1982), and (c) the creation of a separate species for *E. scabrus* var. *plurinervis*, possibly *E. plurinervis*, as its hybrids with *E. scabrus* are sterile (Carman, unpublished). An effort to revise these taxa is currently underway at the Australian National Herbarium, Canberra, ATC, by Murray Hinwood and Judy West.

Elymus rectisetus is recognized in the field (or greenhouse) by its short glumes, long awns, relatively long rachis internodes, and generally low fertility; *E. scabrus* is recognized by its short awns, short glumes and relatively short internodes, *E. scabrus* var *plurinervis* is recognized primarily by its large glumes and secondly by its long awns. Detailed natural distribution, self fertilization, inbreeding depression, and interspecific hybridization studies involving *E. rectisetus* and/or its close sexual relatives have been conducted (Connor 1954, 1962a, 1962b, Hair 1966, Love and Connor 1982, Torabinejad et al. 1987).

Elymus scabrus and *E. rectisetus* share the same 3 basic genomes. Hybrids between them are male sterile but produce PMCs with near perfect pairing (21 bivalents, mostly rings), though reduced pairing (as low as 9 bivalents) are common when certain genotypes of *E. rectisetus* are used as parents (Connor 1962a, Crane and Carman unpublished). Genome analyses indicate that these taxa contain the Y genome of the near eastern *E. longearistatus* (Torabinejad et al. 1987), somewhat modified versions of the S and Y genomes of *E. semicostatus*, and the Australasian W genome from *Australopyrum* (Torabinejad, unpublished).

Intergeneric hybridization with the Australasian Elymus hexaploids has been successful. Love and Connor (1982) hybridized this group with *Critesion* and *Pseudoroegneria* species. Torabinejad (1988-1989, personal communication to J.G.C. and R.R-C.W.) hybridized *E. scabrus* with *Secale montanum*, *Cretesion californicum*, *Australopyrum retrofractum*, *Psaphyrostachys juncea*, and *Thinopyrum bessarabicum* and *E. rectisetus* with *Pseudoroegnaria spicata* and *Hordeum vulgare*. Ahmad and Comeau (1991) hybridized *Triticum aestivum* with the sexual *E. scabrus* var. *plurinervis*. Liu, Wang and Carman (1990, unpublished) hybridized T. aestivum (two lines) with apomictic *E. rectisetus*. These latter $2n=9x=63$ F_1s contain the complete genomic constitution of *E. rectisetus* (42 chromosomes) in the wheat cytoplasm. A BC1 to wheat has also been produced, which contains 54 chromosomes (discussed below). The F_1s and the BC_1 regenerate readily from

embryogenic callus, which is readily induced from immature inflorescences.

Megasporogenesis. The initial cytologically-detectable diversion from normal megaspore mother cell (MMC) development in *E. rectisetus* is the formation of vacuoles in the chalazal end of the MMC, which occurs during early prophase (Crane and Carman 1987). This phenomenon is temporally associated with a lack of callose deposition within and about the cell walls of the MMC. Such deposition consistently occurs during this time in sexual MMCs within this species (Carman et al. 1991) and in other angiosperms with monosporic or bisporic embryo sac development (Rodkiewicz, 1970).

By leptotene the nucleus of apomeiotic *E. rectisetus* MMCs becomes enlarged and slightly elongated, and moves to the center of the MMC. This stage is of normal duration in about 40 to 50% of *E. rectisetus* ovules (varies widely by accession). In such ovules the MMC advances rapidly toward apomeiotic division, embryo sac development, and presumably normal ovule fertility. In the remaining ovules, this stage of nuclear positioning and elongation is of an abnormally long duration. In such ovules the nucleus becomes grossly elongated, occasionally assuming a bell-bar shape, and vacuoles enlarge, shrink and disperse rapidly (Crane and Carman 1987). There is scant evidence suggesting that those MMCs that enter this prolonged stage eventually form normal embryo sacs. Most are probably abortive, and contribute to low seed set.

The apomeiotic division in *E. rectisetus* is characterized by the nuclear membrane dissolving, the unpaired chromosomes condensing, and karyokinesis, which may or may not be followed by cytokinesis. Thus either a 2*n* megaspore dyad or a binucleate embryo sac forms (Crane and Carman, 1987). Definitive EM studies to determine whether pairing and crossing over occurs have not been conducted. There is weak evidence from light microscopy studies that recombination may infrequently occur (Crane, personal communication). Embryo sac development of the Polygonum type proceeds normally from the surviving apomeiotic megaspore and the mature megagametophyte is structurally identical to that in reduced, meiotic ovules (Hair, 1956; Crane and Carman, 1987). Sexual megasporogenesis occurs infrequently within the spikes of apomictic *E. rectisetus* (0.1% to as high as 5%, depending on the accession) but exclusively in *E. scabrus* (Hair, 1956; Crane and Carman, 1987).

Current Research

Cytological techniques. Diplospory in *E. rectisetus* is confirmed cytologically by deviant megasporogenesis, i.e., vacuolate young MMCs, elongated MMC nuclei, near absence of callosic MMC walls, and the relative absence of metaphase one and linear tetrads. Most of these traits are best observed using Nomarski (interference) contrast microscopy of cleared pistils whose corresponding anthers contain meiotic PMCs (Crane and Carman 1987). We routinely clear properly staged pistils (staged by a quick examination of PMCs) by sequential transfer (5 to 10 min in each clearing solution) from Carnoys fixative to 70% EtOH (for storage), to 95 % EtOH, to 95 % EtOH : benzyl benzoate 2:1 (v:v), to 95 % EtOH : benzyl benzoate 1:2 (v:v), to benzyl benzoate : dibutyl phthalate (teratogen, avoid contact with skin) 2:1 (v:v). Several ovules are then placed on a slide and are held in sagittal section between two cover slips. A third cover slip covers the pistils and a droplet of clearing solution is allowed to fill the spaces between the pistils by capillary action. Excellent clearings are produced, though resolution is improved when pistils are cleared more slowly (Crane and Carman 1987).

Callose in the walls of MMCs, sexual dyads and tetrads is detected by florescence when paraffin-sectioned pistils are stained for 15 min in an aqueous solution containing 136 ÊM aniline blue (Fisher Chemicals) and 50 mM K_2HPO_4 (pH'ed to 9.5 with K_3PO_4) and exposed to UV microscopy (Carman et al. 1991). Sectioning requires several days of preparation. We have recently developed a clearing medium that contains 136 ÊM aniline blue, 50 mM K_2HPO_4 and 2.46 M sucrose (pH'ed to 9.5 with K_3PO_4). Entire pistils are cleared by sequential transfer (5 to 10 min each) from 70% EtOH, to 30% EtOH, to half-strength clearing solution, to full strength clearing solution. The quality of clearing is poor, but the clarity of fluorescing MMCs, dyads and tetrads using phase contrast optics in combination with UV florescence is nearly as good as from sectioned material. The major advantage is that numerous ovules can be screened within 1 h for the presence of callosic meiocytes, which is a strong positive indication of sexual megasporogenesis. Nomarski optics are then used to study other cytological details from selected

pistils that are recleared in benzyl benzoate dibutyl phthalate. Recleared ovules fail to show callose florescence.

Genetic analyses of apomixis in E. rectisetus. The transfer of apomixis from *E. rectisetus* to domestic Triticeae will require some understanding of its genetic regulation. Hair produced three $2n=9x=63$ hybrids by crossing sexual *E. scabrus* ♀♀ with a $2n$-pollen-producing apomictic *E. rectisetus* ♂. The F_1 and F_2 generations from one of these plants showed from 4 to 40% pollen fertility and were partially seed fertile. Much variation in chromosome number had accumulated by the F_5 generation, the average being $2n=73$ (Hair 1966, Love and Connor 1982). These data preclude the occurrence of a strictly meiotic or apomeiotic megasporogenesis throughout the five generations. Syngamy of unreduced eggs or sperm with reduced eggs or sperm was probably involved, and might have occurred as a result of segregation for apomixis.

Our studies of $2n=63$ wheat X *E. rectisetus* suggest the presence of wheat nuclear or cytoplasmic alleles that prevent apomixis. Our $2n=54$ BC_1 produces 10% stainable pollen and its PMCs average 12 to 14 bivalents. It almost certainly arose from the union of a reduced egg from the F_1 (21 chromosomes from the 21 pairs of *E. rectisetus* plus 12 wheat chromosomes from the original 21 wheat univalents) and a reduced sperm nucleus (21 chromosomes) from wheat. This interpretation is supported by cytological studies of megasporogenesis. In our Fukohokumuge X *E. rectisetus* F_1 and the BC_1 from this F_1, MMCs and their division products showed strong callose florescence and tetrads were observed. In many ovules, particularly in the F_1, megasporogenesis was in a state of disarray, which may have been caused by hybrid breakdown or by apomixis genes attempting, yet failing, to be expressed. Megasporogenesis in the other F_1s (Chinese Spring X *E. rectisetus*) has yet to be studied.

It is possible that variable expressivity for the apomixis mechanism occurs in certain wide hybrids involving *E. rectisetus*. Pistils from hybrids we produced between *E. scabrus* and *E. rectisetus* were studied by Crane (1988, unpublished) who provided limited evidence that the apomixis mechanism was being initiated but not completed, e.g., MMC prophase nuclei were slightly elongated and vacuoles occurred in the young MMC cytoplasm. Both of these traits are typical of the early stages of apomeiosis.

Future Plans

Our objective is to domesticate apomixis. To do this, we must know how many genes are involved and something concerning their inheritance. To answer these questions we are attempting to produce germplasm that, upon selfing, will segregate for apomixis. The segregant F_2 or BC_1 populations will then be bulked according to reproductive type and tissues will be analyzed using PCR/RAPD methods (Michelmore et al. 1991) to identify markers linked to apomixis genes. We anticipate using approximately 160 primers for RAPD generation. We have assembled a collection of ecotypically and morphologically diverse germplasm for this purpose. It consists of $2n$ and n-pollen-producing accessions of apomictic *E. rectisetus* from Australia, New Zealand and Tasmania; sexual *E. scabrus* and *E. scabrus* var. *plurinervis* from Australia; and sexual *E. scabrus* from Australia with morphological characteristics that suggest natural introgression between apomicts and sexuals. To date, several crosses have been made.

Many hybrids involving genetically-diverse lines may be required to obtain germplasm that either expresses or segregates for apomixis. This will likely also be true in our attempts at transferring apomixis to wheat. Such is consistent with the apomixis literature, and was experienced by Hanna with millet and by the Russians with *Zea maize* X *Tripsacum dactyloides*. Pursuant to these circumstances is an intensified effort on our behalf to produce additional F1s and BC1s using diverse wheat and *E. rectisetus* germplasm.

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Transfer of Apomixis in *Pennisetum*

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Introduction

There are few genes, if any that could have a greater impact on improving the quality and quantity of world food, feed, and fiber production than the gene(s) controlling apomixis. For this reason we have dedicated a large portion of a research effort to: 1) demonstrate that gene(s) for apomixis can be transferred from wild to cultivated species, 2) identify gene(s) controlling apomixis that could be cloned and used in other genera to fix hybrid vigor and produce true-breeding hybrids, and 3) develop apomictic or true- breeding pearl millet [*Pennisetum glaucum* (L.) R. Br.] hybrids. Some current methods being tested for transferring gene(s) controlling apomixis include: 1) natural recombination, 2) chromosome substitution, 3) gamma irradiation of pollen and cell suspensions, 4) somatic variation of plants from cell culture, and 5) DNA transfer.

Pearl Millet Mutants

Our interest in utilizing apomixis to produce true-breeding hybrids in pearl millet (*Pennisetum glaucum*) began in the early 1970s with the discovery of a radiation-induced stubby head facultative apomictic mutant (Hanna and Powell, 1973). The multi-ovary, -ovule, -embryo sac mutant averaged 26% maternal types based on test crosses. At about the same time, another radiation induced female sterile (fs) but male fertile mutant was discovered (Hanna and Powell, 1974). The fs mutant produced multiple aposporous-type embryo sacs but set no seed. Later studies showed that fertilization only occasionally takes place and when it does, embryos and endosperm abort presumably due to limited nucellar tissue in the ovules (Arthur et al., 1991 and unpublished). Preliminary data indicate that the stubby head and fs mutants are allelic and represent steps in the process from sexual to apomictic development in pearl millet. These mutants are allowing us to investigate the developmental steps in the apomictic process and we are using them to develop new mutants representing other steps (unpublished).

Interspecific Gene Transfer

The obligate apomictic *Pennisetum* species used in our gene transfer program included *P. setaceum* (2n = 3x = 27), *P. orientale* (2n = 4x = 36) and *P. squamulatum* (2n = 6x = 54). All belonged to the tertiary gene pool (Hanna, 1987a) according to the classification of Harlan and deWet (1971).

Sexual secondary gene pool *P. purpureum* (2n = 4x = 28) was used as a bridging species to improve fertility of interspecific hybrids. Diploid (2n = 2x = 14) pearl millet was used as female parent in the first hybrids. Tetraploid (2n = 4x = 28) pearl millet was later used in the interspecific crosses to improve male fertility since apomixis in our crosses was expressed as a dominant character and therefore needed to be transferred through the pollen. The efficiency of our backcrossing program was enhanced by improving the seed set on tetraploid pearl millet (Dujardin and Hanna, 1989a).

Pearl millet x *P. setaceum*

P. setaceum (2n = 27) was the first apomictic species we crossed with pearl millet because of its low chromosome number and high pollen fertility. Hybrids (2n = 25) with diploid pearl millet (2n = 14) were completely male sterile and highly female sterile but showed apomictic development (Hanna, 1979). No hybrids were obtained with tetraploid pearl millet (Dujardin and Hanna, 1989b). This interspecific cross did not appear to have potential for apomixis gene transfer because of complete male sterility.

Pearl millet x *P. orientale*

P. orientale was the second apomictic species we crossed with diploid pearl millet because we felt the autotetraploid nature of this species would allow us to introduce two sets of chromosomes with gene(s) for apomixis, thereby improving male fertility. Sexual, facultative apomictic, and highly apomictic hybrids with 2n = 25 chromosomes were obtained (Hanna and Dujardin, 1982). The 25- chromosome interspecific hybrids were male sterile with high female sterility. Seed produced on these hybrids from pollinations with diploid pearl millet pollen produced 23-, 27-, and 32-chromosome backcross-1 plants. Although the 27-chromosome plants were obligate apomicts, they were of little value for further backcrossing because they were male sterile. The 23- and 32-chromosome plants were facultative apomicts with only a low frequency of apomixis (Dujardin and Hanna, 1983a and 1987b). Male fertility was improved in male fertile tetraploid (2n = 4x = 28) pearl millet x *P. orientale* hybrids but the hybrids were of little value in a backcrossing program because of the low frequency of apomictic expression. An important finding with these backcrosses was that

polyploidy was not necessary for expression of gene(s) controlling apomixis since the 23- chromosome plants had only one (simplex) set of (or nine) *P. orientale* chromosomes.

Pearl millet x *P. squamulatum*

P. squamulatum proved to be an excellent donor of gene(s) controlling apomixis to pearl millet. Interspecific hybrids (2n = 41) were: 1) relatively easy to produce using tetraploid pearl millet as female parent, 2) highly apomictic and 3) highly male fertile (Dujardin and Hanna, 1983b and 1989b). Pollen from apomictic interspecific hybrids used to pollinate tetraploid pearl millet resulted in numerous sexual and apomictic progeny but most were male sterile or highly female sterile (Dujardin and Hanna, 1985a).

Progress in transferring of gene(s) controlling apomixis was greatly enhanced with improved pollen fertility (up to 94%) of trispecific hybrids from sexual 42-chromosome pearl millet - *P. purpureum* interspecific hybrids x 41-chromosome apomictic pearl millet - *P. squamulatum* crosses (Dujardin and Hanna, 1984a). *P. purpureum*, an allotetraploid with the A' and B genomes, proved to be an important bridging species in the gene(s) transfer program. Pollen from apomictic trispecific hybrids were used to pollinate tetraploid pearl millet to produce an apomictic BC_1 plant with 45% stainable pollen. Tetraploid pearl millet x apomictic BC1 crosses produced 29- to 35-chromosome BC_2 plants with up to 28% pollen stainability. BC_2 plants backcrossed to tetraploid (2n = 28) pearl millet produced an obligate apomictic 29-chromosome BC3 plant with 37% stainable pollen (Dujardin and Hanna, 1989c). Another backcross to pearl millet has produced 27-, 28- and 29-chromosome apomictic BC4 plants (unpublished). Morphologically, plants in each succeeding backcross generation appear to more closely resemble pearl millet.

Conclusions

The research to date has shown: 1) transfer of apomixis to pearl millet is possible by using: a) bridging hybrids between pearl millet, *P. purpureum*, and *P. squamulatum*, b) tetraploid pearl millet as female recurrent parent, and c) selected backcross derivatives with pollen fertility and obligate apomixis as pollen parent; 2) apomixis was as strongly expressed in advanced as in early backcross generations; 3) obligate apomicts were less frequent in advanced backcrosses because of lower transmission frequency of the extra chromosome(s) with gene(s) controlling apomixis; 4) obligate apomictic BC_4 plants have been recovered; 5) most chromosomes of *P. purpureum* (the bridging species) and *P. squamulatum* (apomixis gene(s) donor species) have been eliminated after four backcrosses. Research has shown that large populations were necessary to recover the needed apomictic progeny with some male fertility. Most apomictic plants have been later maturing than the tetraploid sexual pearl millet recurrent parent at this point in the backcrossing program. The methyl salicylate clearing technique has been extremely useful for identifying apomictic backcrosses before they finished flowering.

Future Research

Future research will involve: 1) continue backcrossing and selecting for pearl millet-like apomictic plants to produce an apomictic pearl millet, 2) increase genetic diversity of tetraploid pearl millet used as recurrent parent, 3) identify *P. squamulatum* chromosomes(s) or chromosome segments associated with apomixis, 4) study steps in the apomictic process, 5) study genetics of apomixis, 6) develop molecular probes linked with gene(s) controlling apomixis, and 7) clone gene(s) controlling apomixis for use in other plant genera.

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Molecular Research on Apomixis in *Pennisetum*

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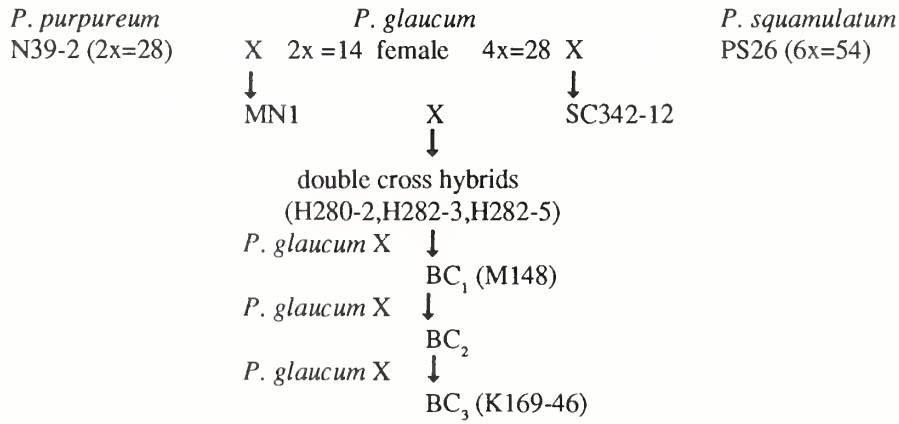
Apomixis in the genus *Pennisetum* and progress towards transfer of the trait for apomixis from the wild species, *P. squamulatum*, to pearl millet (*P. glaucum*) have been reviewed by Wayne Hanna et al. (USDA Apomixis Workshop 1992). The production of an apomictic backcross plant (BC_3), with some of the wild germplasm contributing the genes controlling apomixis, provided the opportunity for us to contemplate the application of molecular techniques to understanding the genetic control of apomixis in *Pennisetum*. The pedigree of BC_3 is shown in Fig. 1.

The backcross 3 plant is an obligate apomict and has 29 chromosomes. This led us to assume that 28 of the chromosomes were from pearl millet and a single chromosome was from *P. squamulatum*. The molecular data do not support this assumption.

DNA was isolated from young leaves of BC_3 and used to prepare a DNA library. The restriction enzyme, *Pst*I, was used to cut the DNA because of its previously demonstrated ability to preferentially cut in regions of single copy DNA. *Pst*I-digested BC_3 DNA was ligated with pUC19 vector DNA, and the recombinant plasmids were cloned into host bacterial strain DH5alpha. Approximately 90% of the *Pst*I clones have proved to be single to low copy number.

Genomic DNA was isolated from all parents of BC_3: pearl millet genotypes, 23BE and 239DB; Napier grass introduction, N39-2; and *P. squamulatum* accession, PS26. DNAs of BC_3 and all four parents were digested with several restriction enzymes, separated on an agarose gel, blotted onto nylon membranes, and hybridized with randomly chosen *Pst*I clones. Although the techniques are relatively straightforward, they require a considerable amount of time and money to perform. However, DNA markers have an advantage over phenotypic or isozyme markers in that they are potentially more numerous and are not a result of gene expression which can change with environment. By applying Southern blot hybridization to the analysis of genomic DNAs, it was possible to detect restriction fragment length polymorphisms (RFLPs), when they existed, between all parents that were included in the pedigree of BC_3. More importantly, restriction fragments that were shared only by BC_3 and its apomictic parent, i.e., also absent from all the sexual parents, indicated inheritance of genes from the apomictic parent, *P. squamulatum*. After surveying 48 *Pst*I clones, 7 of them were informative. One of these clones (197) appeared to be species- specific by Southern blot analysis. Clone 197 hybridized only with BC_3 and *P. squamulatum*. The DNA sequence of clone 197 was determined and primers were designed which would amplify this sequence using the polymerase chain reaction (PCR). PCR amplification with the 197 primers resulted in the expected 140 bp band in *P. squamulatum* and BC_3, which was absent from the sexual parents. Furthermore, the 197 primers also amplify the same piece of DNA in many other *Pennisetum* and closely related apomicts including but not limited to *P. orientale*, *P. setaceum*, *P. flaccidum*, *P. villosum*, and *Cenchrus ciliaris*.

Fig. 1. Pedigree of BC_3 (Dujardin and Hanna 1989).



Another technique for detecting DNA polymorphism is called RAPD (random amplified polymorphic DNA) or AP-PCR (arbitrarily- primed PCR). This method uses a single primer, usually 10 bases long, in the polymerase chain reaction, to amplify several regions of targeted DNA. We have surveyed BC_3 and its parents with 78 primers and frequently detect polymorphism among all four parents. Only 5 of the 78 primers have amplified a reproducible band that was shared only by BC_3 and *P. squamulatum.*

A backcross 4 population was produced by pollinating male sterile tetraploid pearl millet with pollen from BC3 plants. Male sterile pearl millet was used because the pollen fertility of BC3 is relatively low and crosses onto male fertile pearl millet always result in a significant number of selfed progeny. The only way to ensure that BC4 progeny resulted from fertilization with BC3 pollen was to use a male sterile line as the female parent. Only 2 out of 77 BC4 progeny were obligate apomicts, 4 were facultative apomicts, and the remaining were sexual. Thus far, 25 sexual and 2 apomictic male sterile BC4 progeny along with a limited number of progeny from 5 male fertile apomictic BC4s have been screened with 4 molecular markers previously identified as informative. We have observed that the progeny fall into two groups. One group contains a *P. squamulatum*-specific RAPD marker from 2 different RAPD primers. This group represents 48% of the male sterile BC4 progeny surveyed and the RAPD marker appears in both sexuals and apomicts. The second group contains one *P. squamulatum*-specific RAPD marker and the 140 bp fragment amplified by the 197 primers. This group contains only apomictic BC4 progeny.

Finally, the 140 bp fragment also is amplified in the apomictic F_1 of *P. glaucum* x *P. squamulatum* (SC342-12; Fig. 1) but not in a sexual F1 plant derived from the same cross. This suggests that *P. squamulatum* (PS26) is heterozygous for apomixis (Dujardin and Hanna 1983) and the presence of the 197 DNA sequence.

Heterozygosity for the molecular marker might allow genetic linkage to be established in a population of interspecific F_1 hybrids.

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Searching for Apomixis in Rice

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Introduction

Hybrid rice, utilizing the 3-line cms system, is grown on more than 10 million hectares in China. Because of labor-intensive hybrid seed production techniques and unsuitable grain quality, virtually no hybrid rice is grown elsewhere. Since apomixis, or asexual seed production, theoretically can be used to produce true- breeding F1 hybrids with permanently fixed heterosis (Bashaw, 1980), a search for this characteristic in rice was conducted at Davis, CA from 1985-88 (Rutger et al., 1986; Rutger, 1988). Four approaches were considered:

1. Screening for apomixis within cultivated rice (*Oryza sativa*). This technique is dependent upon finding maternal-type plants among or instead of F1 hybrids and upon finding F1 hybrids which fail to segregate normally (Bashaw, 1980).

2. Screening for apomixis in related wild species of the crop. This technique is dependent upon cytological identification of apomictic embryo sacs in the wild species (Young et al., 1979)

3. Intergeneric hybridization with known apomicts.

4. Genetic engineering.

Results & Discussion

Approach 1. Screening for apomixis within cultivated rice.

a. Aberrant F2 plant segregation

Progenies of 3,728 F1 plants, resulting from natural crossing of an M-101 genetic male sterile with about 400 world collection rices, were examined in the 1985 nursery. These F2 populations were scrutinized for abnormal segregation of three marker genes: tall (Sd) vs. semidwarfism (sd), pubescent (Gl) vs. glabrous (gl) leaves, and fertile (Ms) vs. male sterile (ms). The female parent was homozygous recessive for all three markers. Because of the large number of populations involved, average F2 population size was only 29 plants. Of the 3,728 lines, 87 produced an excess of F1 parent types at the 0.05 probability level, and 7 at the 0.01 level. This abnormal segregation for an excess of F1-parent types was a possible indication of apomixis.

For these 94 lines, the F2 plants bearing the F1 phenotype (tall, pubescent, fertile) were progeny tested in 1986. From 9 to 20 rows (each from a separate F2 plant), at a minimum of 20 plants per row, were grown. Only 16 of the 94 families deviated from the expected 2 heterozygous: 1 homozygous ratio for one or more marker genes. Two families again showed no segregation, i.e., all plants were of the F1 parent type. However, in embryo sac analysis none of these plants showed any signs of apomictic or otherwise abnormal embryo sacs. Apparently these two families arose from an inadvertent mechanical mixture in 1984. Of the other 14 unusual families in 1986, 12 showed abnormal segregation for one marker gene, and two for two marker genes. Since none of these 14 families displayed the heterozygous condition for all three markers, it was concluded that the abnormal segregation ratios in the 1985 generation were caused by sampling error due to small sample size, not apomixis.

b. Aberrant F1 seed segregation

Two genetic male sterile stocks, M-101 #2 *msms wxwx* and M-201 Np *msms wxwx*, were used as females in natural crossing blocks with some 400 normal translucent endosperm (*MsMs WxWx*) males in 1986. The waxy endosperm marker permits the identification of seeds resulting from hybridization; all such seeds are of the genotype *Wxwx* and are translucent in appearance. The presence of *wxwx* seeds, which are opaque, would be a possible indication of apomixis in the female. Unfortunately, all 19,719 seeds harvested from the waxy male sterile females proved to be true crosses, indicating the absence of apomixis in the two females.

A spin-off of this study was the identification of two genetic male steriles, M-101 ms and M-201 Np ms, as useful lines for population improvement studies since they showed relatively high rates of outcrossing (ca 20%) (Hu and Rutger, 1991, 1992). Another related spin-off was the identification of an environmentally-influenced genetic male sterile (Oard et al., 1991). Such environmentally-influenced genetic male steriles may be useful for 2-line hybrid rice (Rutger, 1991).

c. Aberrant F1 plant segregation

At a 1986 Bellagio conference on apomixis, it was recommended that several thousand world collection lines be used

as females in crosses with a dominant purple leaf marker line. In such crosses, the presence of green F1 seedlings would be an indication of apomixis (or of selfing). Following the conference 367 world collection lines (as females) were crossed with the dominant purple leaf line PI408449. In 366 crosses, all F1 seedlings were purple, with no indication of apomixis. In one cross, PI 439045/PI 408449, 25 purple and 10 green seedlings were found. All seedlings, both purple and green, were grown to maturity and progeny tested. Progenies of the purple plants segregated for leaf color as would be expected of true F1s. No segregation occurred among progenies of the green plants. However, embryo sac analysis did not show any abnormalities, so the original 10 green seedlings were concluded to be the result of selfing, rather than apomixis.

d. High frequency twinning

High-frequency twin seedling occurrence, a possible indication of apomixis, was studied on lines brought from China by Mr. Li Yuan Ching (Mr. Yuan Long-Ping's assistant). An as-yet unpublished manuscript, "Characterization and inheritance of twin seedlings in rice (*Oryza sativa* L.)", by Li Yuan Ching et al., (199_), has been prepared. Briefly, four rice lines, designated AP I, AP II, AP III, and AP IV, exhibited twin seedling rates of 16.1, 23.4, 32.4, and 5.0 percent, respectively. Twin seedlings were of two types, one-mesocotyl and two-mesocotyl. Embryo sac analysis indicated that most twin seedlings originated from fertilized two-egg embryo sacs or multiple-egg embryo sacs. Evidence for apomixis was not conclusive.

Approach 2. Screening for apomixis in related wild species of rice.

Dr. Gurdev Khush graciously grew 547 accessions, of 13 wild species of *Oryza*, at IRRI during the period 1985-87.[1] Jinguo Hu, predoctoral fellow on the project, travelled to IRRI for a month in January, 1986, and again in January, 1987, to collect pre-anthesis florets of these accessions and bring them to Davis for embryo sac analysis.

The normal mature rice embryo sac contains one egg cell, two synergids, two polar bodies and three antipodals. At the time of sampling the antipodals had divided several times so only cell masses in the antipodal position were observed. Any unusual appearance of the embryo sacs was considered as an abnormality. Although no clear evidence of apomixis was found in the accessions, the pistil-clearing method revealed one or more abnormal embryo sacs in 68 accessions. A total of 175 abnormal embryo sacs was observed:

a. One or more extra nuclei within the embryo sac	145
b. Extra cell or cells within the embryo sac	22
c. An embryo sac within the embryo sac	6
d. Two separate embryo sacs within one pistil	2

From among the 68 accessions bearing abnormal embryo sacs in 1985 or 1986, 22 were grown in a second test, in 1987. Only nine showed abnormalities in the second test (Table 1). In 1988 these nine lines were imported to the plant quarantine nursery at Beltsville, Maryland. Six lines failed to germinate. Following the untimely death of the Beltsville curator in mid 1988, the other three lines were inadvertently discarded. By that time the author had left California for his present assignment, so the materials were not re-imported. Whether any of the observed abnormalities were due to apomixis is unclear. Also unclear is whether the low frequencies would be of value even if the cause was apomixis. Nevertheless, these nine lines merit further examination.

Approach 3. Intergeneric hybridization with known apomicts.

Since earlier reports from China indicated that rice x *Pennisetum* crosses were possible, and since several *Pennisetum* species are apomictic, such crosses were attempted. In 1986-87, four different rice genotypes were pollinated with *Pennisetum setaceum*, an apomictic pearl millet species supplied by Dr. Wayne Hanna, USDA-ARS, Tifton, GA.[2] Some *Pennisetum* pollen germinated on the rice stigmas, so 978 rice florets were pollinated. Embryo rescue techniques were used to culture 600 ovaries in 1986-87; 351 of the ovaries exhibited swelling but none developed into mature seed.

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[1]Appreciation is expressed to Dr. Gurdev S. Khush for his cooperation.

[2]Appreciation is expressed to Dr. Wayne Hanna for his cooperation.

Table 1. Embryo sac abnormalities in 9 accessions grown in two tests.

IRRI Accession Number	Species	No. embryo sacs in first test		No. embryo sacs in second test	
		Total	Abnormal	Total	Abnormal
104448	O. sativa/nivara	34	2	55	3
101979	O. nivara	30	2	48	2
100649	O. perennis	29	2	53	2
104131	O. barthii	68	2	70	2
105173	O. officinalis	61	1	42	1
104116	O. barthii	51	3	52	1
101383	O. longistaminata	32	1	56	1
101988	O. nivara	33	3	63	1
100117	O. barthii	98	3	46	1

In 1988, 11,036 rice florets were pollinated with pollen from two other known apomictic pearl millet species, *Pennisetum flaccidum* and *Pennisetum squamulatum*, but no true hybrid seeds were formed.

Approach 4. Genetic engineering.

A suspension culture protocol was developed, but no further progress was made.

Summary

In screening for apomixis within cultivated rice, four types of experiments were conducted: screening for aberrant F2 plant segregation (none confirmed), for aberrant F1 seed segregation (none found), for aberrant F1 plant segregation (none confirmed), and for high-frequency twinning. The high-frequency twinning study documented four lines from China with twinning rates of 16.1, 23.4, 32.4, and 5.0 percent. Embryo sac analysis indicated that most twin seedlings originated from fertilized two-egg or multiple-egg embryo sacs. Evidence for apomixis was not conclusive.

In screening for apomixis in wild species of *Oryza*, 547 accessions, representing 13 species, were grown by IRRI cooperator Dr. Gurdev Khush. Embryo sac analyses, conducted in California, revealed nine accessions that showed low frequencies of abnormalities over two growing seasons. It was not clear whether these abnormalities indicated apomixis.

In attempts to make intergeneric hybrids with known apomicts, some 12,000 rice florets were pollinated by *Pennisetum* species, but no crosses were recovered.

Attempts to use genetic engineering techniques to transfer apomixis resulted in development of a rice suspension culture protocol, but did not progress further.

Spin-offs of the research included the identification of two genetic male steriles useful for population improvement studies, and of an environmentally-influenced genetic male sterile which may be useful in 2-line hybrid rice.

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Apomixis in Sorghum

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Facultative apomixis at a variable frequency has been discovered in cultivated grain sorghum, *Sorghum bicolor* (L.) Moench but is not yet useful. Apomixis has been reported in seven lines, the most promising being line R473 from India. Research is in progress in India and the USA to perfect apomixis in R473 and to identify other promising sources.

Sorghum bicolor, a functional diploid with 2N=20 chromosomes, is a self pollinated species with perfect florets. Sorghum has a polygonum type embryo sac and normally reproduces sexually. Hybrids have a high level of hybrid vigor and have essentially supplanted pure line cultivars in the US and in some other countries where sorghum is grown. The mass production of F1 hybrid seed depends on the cytoplasmic-nuclear male sterility of the female parent. Because of the complexity of that type of seed production, not all countries, including many in which sorghum is an important food crop, produce hybrid sorghum, relying on self-pollinated cultivars instead.

Apomixis was first reported in 1968 in line R473 in India (35) and has since been reported in six additional lines (2, 28, 42). Aposporv is the mode of apomixis reported for those lines in which the mode has been determined. The frequency of apomictic seed formation is less than 25 percent except for R473 for which maternal offspring have been reported to be sometimes as frequent as 100 percent.

R473 is the line most studied (27) for apomixis. In the original report (35) it was indicated that in R473 a nucellar cell develops into an embryo sac. An additional unusual characteristic of R473 is that is cross sterile (21) setting very few seed when emasculated and pollinated by other lines. Its own pollen, however, functions quite well in fertilizing itself or other lines. We have observed (unpublished) that pollen of other lines does not germinate well and has abnormal tubes when placed on stigmas of R473.

The cross sterility of R473 has made it difficult to determine the exact frequency of apomictic seed formation because the usual type of progeny test is precluded. Observations of isozyme genotypes of progeny from selfing (6) have provided evidence that R473 is not an obligate apomict. Plants with diverse isozyme genotypes were observed in progeny from selfing. Induced mutations within R473 have been used to determine frequency of apomictic seed formation. In one study (37) induced malesterile plants in R473 were crossed with normal plants of R473 and no evidence of apomictic seed formation was detected. Other studies (18, Murty, unpublished) using other induced genetic markers such as bloomless, shrunken endosperm, and plant color, have provided a range of results from 0 to 100 percent maternal offspring. Unfortunately, selection of those with the highest percentages have not fixed apomixis at a higher level. Nor has selection separated apomixis from cross sterility.

Other lines in which apomixis has been reported include a polygynaceous line (2). Multiple embryo sacs were observed to be frequent in that line. Multiple embryo sacs have been observed in other lines as well (42). Among these are two lines that were derived in a program in which seedlings were treated with colchicine and mutant types were derived. The highest frequency of maternal offspring from these lines was 20 percent. Apomixis was recorded for a hybrid between two wild types of sorghum (28).

Several obstacles remain to making apomixis useful in sorghum. None of the lines for which apomixis has been reported has a high and stable frequency of apomixis. In addition, R473, the line for which the highest frequency has been reported, is cross sterile, making it difficult to manipulate in a breeding program.

Current research and plans include: (1) Use of molecular markers to determine more precisely the frequencies of maternal offspring from plants of R473. (2) Separation of apomixis from cross sterility in R473, (3) Search for new sources of apomixis, especially among wild species, (4) Molecular tagging of the genes for apomixis so as to improve the efficiency of selection.

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Apomixis in *Tripsacum*

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Tripsacum L. is allied with the genus *Zea* in the subtribe *Tripsacinae* of the *Andropogoneae* tribe of the *Gramineae* (Poaceae). It is the only genus known to produce viable hybrids when crossed with maize (*Zea mays* L. spp. mays). *Tripsacum* is a complex genus, encompassing at least 16 species which range from 42 °N to 24 °S latitude in the new world. *T. dactyloides* (L.) L is by far the most variable species, occurring throughout the range of the genus, being the only species to extend into temperate climates of the U.S.

Tripsacum species have been grouped into two supraspecific groups recognized as sections. General section systematics, chromosome numbers and distribution are as follows:

Section *Tripsacum*: Terminal inflorescences with 1-10 (rarely more) digitately arranged branches that are never pendent; both spikelets of a staminate pair sessile; lower glume of staminate spikelet coriaceous.

***Tripsacum* spp.**	**2n**	**Distribution**
T. andersonii	64	Tropical Americas
T. australe	36	South America
T. bravum	36,72	Mexico
T. cundinamarce	36	South America
T. dactyloides	36,54,72,90, 108	42°N to 24°S Lat. S. Florida and Cuba
T. floridanum	36	Mesoamerica
T. intermedium	72	Mesoamerica
T. latifolium	36	Mexico
T. manisurodies	36	South America
T. peruvian	72,90,108	Mesoamerica
T. zopilotense	36,72	

Section *Fasciculata*: Terminal inflorescences with usually more than 10 branches, with male section becoming pendent; one of each pair of staminate spikelets supported by a slender, 2-6 mm long pedicel; lower glume of staminate spikelet membranaceous.

***Tripsacum* spp.**	**2n**	**Distribution**
T. jalapense	72	Guatemala
T. lanceolatum	72	S. Arizona - Mexico
T. laxum	36	Mexico
T. maizar	36,72	Mesoamerica
T. pilosum	36,72	Mesoamerica

Tripsacum sp. with 2n=36 behave cytologically as functional diploids, but the high base number, n=18, suggests they are polyploids of ancient origin. Anderson (1944) speculated that 2n=36 cytotypes are probably tetraploid amphidiploid derivatives of distantly related *Tripsacum* diploids, and that 2n=72 cytotypes are octaploid allopolyploids. Galinat (1965) suggested that *Tripsacum* originated as a possible amphidiploid from crosses between wild maize (n=10) and a species of *Manisures* (n=9) with subsequent loss of a wild maize chromosome. Tantravahi (1968) proposed that all tetraploid species of *Tripsacum* originated as segmental allopolyploids from related diploid species. Multiple character analysis and chromosome studies were used to identify *T. lanceolatum* and *T. pilosum*, 2n=72, as intermediates resulting from crosses of *T. zopilatense* and *T. maizar*, 2n=36, (Tantravahi 1971).

Farquharson, (1955) reported 2n chromosome numbers of 36, 45, 54, 72 and 108 arising from tetraploid strains of *T. dactyloides*. Polyembryony was not found in diploid *Tripsacum*, but facultative, pseudogamous apomixis was believed to occur in tetraploid germplasm and polyembryony was common in those strains. Farquharson presented no evidence concerning the mechanism responsible for the presumed apomictic reproduction. Brown and Emery (1958) studied the embryo sac development of a single 72-chromosome clone and reported that it was diplosporous and the embryos had a parthenogenetic or apogamatous origin. In the USSR, Laikova (1976) observed megasporogenesis and embryo sac development in a 72-chromosome eastern gamagrass plant and maize X eastern gamagrass hybrids. Although the actual mechanism of apomixis was not mentioned, the information given for eastern gamagrass was a description of diplospory.

Burson et al. (1990) presented a more complete report on megasporogenesis and embryo sac development in diploid, triploid and tetraploid germplasm of *T. dactyloides*. The triploid and tetraploid accessions were apomictic. In these accessions the megaspore mother cell enlarged: but rather than undergoing meiosis, the cell remained meiotically inactive. The only apparent changes were continued cell elongation and vacuolation. Eventually the nucleus of the elongated megaspore mother cell divided mitotically and subsequently produced an 8-nucleate embryo sac, which appeared similar to a Polygonum type sac. The embryo developed parthenogenetically. Pollination or fertilization

was necessary for endosperm development, which indicates pseudogamy. Diploid accessions reproduced by normal sexual means. After the first mitotic division, there was no difference in appearance between the apomictic and sexual embryo sacs. The results confirmed that the apomictic mechanism in eastern gamagrass was diplospory followed by pseudogamy and that diploids are sexual while naturally occurring polyploids are apomictic.

Hybrids between Maize, *Z. mays* , 2n=20 Zm, and *T. dactyloides*, 2n=72 Td, resulted in F_1 hybrids with 10 Zm and 36 Td chromosomes. The hybrids were male sterile, but female fertile and when pollinated with maize, 2n = 20, reproduced both sexually and by gametophytic apomixis (de Wet et al., 1973). Maize and diploid *T. dactyloides* F_1 hybrids with 2n=28 (10 Zm + 18 Td) when backcrossed to maize resulted in the majority of progeny with 2n=28, through development of unfertilized and unreduced egg cells by means of apomixis (Borovskii, 1970). Newell and de Wet (1974) obtained dihaploids, 2n=28 (10 Zm + 18 Td) from F_1, 2n=56 (20 Zm + 36 Td) backcrossed to maize through reproduction of female gametes by apomixis.

T. dactyloides Diploid (2n=36) x Tetraploid (2n=4x=72) Crosses

Hybrids produced from crossing sexual diploids x Tetraploids as pollen parents were extremely variable. Chromosome counts of 47 selected F_1 hybrids revealed only triploids (2n=3x=54). Seedset of 348 F_1 hybrids produced from (2n=36) x (2n=4x=72) crosses ranged from 0-72% with a mean of 7%. Over one-half of the F_1 hybrids failed to set seed, two-thirds produced less than 10% seed set, and only 3% had seed set above 50%. Mean pollen stainability of the F_1 hybrids was 30% with a range of 0-67%. There was no correlation between percent seed set and pollen stainability.

Four fertile triploids were selected for release as germplasm based on female fertility (seed set), male fertility (pollen stainability) and forage production attributes. Seed set ranged from 53 to 71%, and pollen stainability ranged from 18 to 63% among the four fertile triploids. A high degree of apomictic reproduction is suspected as 96 to 100% of the progeny from open pollinations appeared identical to the maternal parent and 21-41% of their seedlings were twins. These fertile triploid should be useful for the transfer of apomixis and the study of ploidy manipulations (Dewald et al., 1992).

T. dactyloides Tetraploid (2n=4x=72) x Diploid (2n=36) crosses

All tetraploid (2n=4x=72) plants of *T. dactyloides* which we have studied reproduce by facultative apomixis, and none have been encountered with even a moderate degree of sexuality. A range of 85-95% of progeny produced from tetraploids appear identical to the maternal parent. Controlled cross pollinations using tetraploid WW-1008 x diploid pollen parents resulted in eight (2%) off-type progeny. One off-type was found to be pentaploid, 2n=5x=90, through fertilization of an unreduced egg. The other 7 were diploid, 2n=36, and are thought to be dihaploids. The pentaploid was essentially sterile; whereas the dihaploids had good seed set but produced extremely variable progeny by the sexual mode of reproduction.

Inheritance of Apomixis in *Tripsacum dactyloides*

The inheritance of apomixis in *T. dactyloides* is unknown. Because the wild polyploid forms all appear to be highly apomictic and the wild diploid germplasms are obligate sexual, a germplasm development effort is needed. Either obligate sexuality must be transferred to tetraploid germplasm or a high level of apomixis must be transferred to diploid germplasm, before inheritance studies can be initiated.

Although limited efforts have been made to develop sexual-tetraploid germplasm, our primary emphasis has been to transfer apomixis to the diploid chromosome level. We recognize that it has not been possible to develop apomictic-diploid germplasm in some species, however, the large number of chromosomes at the diploid level in this species (36) leads us to suggest that this problem may be more readily overcome in *T. dactyloides* than it has been in some other species. Additionally, the inheritance of apomixis should be simpler and the ratios of apomictic:sexual plants easier to interpret at the diploid than at the tetraploid chromosome level.

This work was initiated by crossing diploid germplasm (2n=2x=36) with a highly apomictic triploid (2n=3x=54) accession, WW-1459, originally collected near La Grange, TX (Sherman et al., 1991). There was a wide range in fertility of the hybrids, but most were highly sterile with 84% having 20% seed set or less. A selected subset of the hybrids that included all the more fertile hybrids and a few of the more sterile hybrids were studied in more detail. These showed the full range of chromosome numbers of the parents, from 2n=36 to 54. If the most apomictic hybrids (determined cytologically) were not considered, an increase in chromosome number resulted in a decrease in fertility ($y=199-4x$, $r^2=0.58$). Although not all highly apomictic hybrids were highly fertile, most were clearly more fertile than their more sexual counterparts of similar chromosome number. The highly apomictic hybrids ranged in chromosome number from 2n=43 to 54 chromosomes, but the most fertile ranged from 45 to 49 chromosomes. In general, hybrids with the most uniform progenies in field plantings came from this highly apomictic group (determined cytologically).

In our current research, four of the highly apomictic hybrids with between 46 and 48 chromosomes were backcrossed to diploid plants. About 1000 hybrids were transplanted to the field in 1991. These backcross hybrids will be evaluated for fertility in 1992. We hope to begin analysis of mode of reproduction in the more fertile hybrids in 1993. Because of the difficulty of cytological analysis of mode of reproduction in *T. dactyloides*, we will use progeny tests for initial evaluation. Later, cytological study of mode of reproduction and chromosome counts of selected hybrids will be needed. We believe we should be able to isolate highly apomictic hybrids with about 40 chromosomes from among these backcross hybrids, with luck we may find a highly apomictic hybrid with as few as 36 to 38 chromosomes.

The approach that resulted in the development of tetraploid-sexual germplasm in Eragrostis curvula, is also being investigated. Progeny of a facultative- apomictic tetraploid accession, WW-1207, were evaluated for off-type (OT) plants. Thirteen plants (2.2%) were identified as definite OT plants and an additional 10 plants (1.7%) were possible OT plants. Progenies from these 23 plants will be evaluated for uniformity in the field and if any are variable, the chromosome number of the parent OT plant will be determined.

Transfer of apomixis from *Tripsacum dactyloides* to *Zea mays*

During the spring of 1991, steps were taken to transfer the genes for apomixis from apomictic eastern gamagrass to maize. Crosses were made in a greenhouse at Temple, Texas between diploid and tetraploid maize lines and highly apomictic triploid and tetraploid eastern gamagrass plants. All crosses were made using maize as the female parent. Seed were recovered from some of the crosses and these are presently beign germinated.

A new cytogenetist position is open at Woodward, OK, which should enhance our efforts to elucidate mechanisms of apomixis in *Tripsacum*.

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Apomixis in *Citrus*

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Apomixis in *Citrus* was reported as early as 1912 by Osawa. It had been observed earlier by breeders but not understood and led to much confusion in the literature. Polyembryony is also common in *Citrus* and its near relatives. Although polyembryony occasionally results from multiple zygotic embryos, it usually results from adventitious or nucellar embryony (Bacchi, 1943). Thus, nucellar seedlings are derived from nucellar embryos and zygotic seedlings are derived from embryos originating from the zygote.

Some authors have assumed that embryos in monoembryonic seeds are necessarily of zygotic origin whether originating from clones that produce only monoembryonic seeds or clones that produce mixtures of mono- and polyembryonic seeds. Some authors have erroneously used the terms nucellar and polyembryonic interchangeably. Nagami kumquat (*Fortunella margarita*) produces only monoembryonic seeds but many are of nucellar origin. Generally, clones that produce only monoembryonic seeds produce only zygotic progeny. Many clones produce mixtures of monoembryonic and polyembryonic seeds. The proportion of monoembryonic seeds does not give a reliable estimate of those from zygotic origin. Hearn (unpublished) found that 23% of seeds from Hudson grapefruit were monoembryonic but all seedlings (409) were nucellar. Apomictic forms of *Citrus* can best be described as facultative but some forms are nearly obligate.

Unlike many apomictic plants that are polyploids, *Citrus* and its near relatives usually have 2N=18 and don't show as much evidence of polyploidy as in other plants.

Taxonomic Implications

Taxonomic classification of *Citrus*, *Poncirus*, *Fortunella*, *Microcitrus*, and *Eremocitrus* as separate genera has been somewhat controversial. There has been less confusion with other genera such as *Severinia*, where genetic barriers have prevented hybridization with *Citrus*. The greatest taxonomic disagreement is at the species level within *Citrus* and apomixis is the cause for much of this disagreement. Parent-progeny trueness-to-type and the inability to hybridize clones have led taxonomists to give hybrids species rank. Tanaka (1954) recognized 159 species of *Citrus*. It is safe to assume that he did not understand apomixis, and he gave species rank to several known hybrids. More recent studies by Barrett and Rhodes (1976) suggest that there are only three valid species within the commercial forms of *Citrus*. Others are of hybrid origin and most have been preserved largely by nucellar embryony and other forms of vegetative propagation.

Environmental and Other Effects

Environmental and internal factors likely affect the proportions of zygotic and nucellar seedlings (Frost et al., 1968). High temperature conditions have resulted in increasing the numbers of zygotic seedlings in polyembryonic cultivars (Nakatani et al., 1978, 1982, 1984). The heated glasshouse had an absolute minimum of 17 to 23°C while the unheated glasshouse had 1-6°C minimum from flower bud stage to the end of flowering. The absolute maximum was about 48°C in each glasshouse. Field-grown trees produced less monoembryonic seeds than even the unheated glasshouse. The proportions of nucellar and zygotic progeny from cross-pollinations were reported by Webber (1900), Frost (1926), and others. Evidence that the pollen parent influences the relative proportions of zygotic and nucellar progeny was reported by Toxopeus (1931, 1936), Torres (1936), Frost (1926), Cameron et al. (1968), and Hearn (1977). It is likely that the wide parental differences result in vigorous hybrid embryos produced. Therefore, some pollen parents cause greater production of zygotic progeny (Frost et al., 1968). Hearn (1977) found that Mediterranean Sweet orange produced up to 100% zygotic progeny when pollinated with *Poncirus trifoliata* and only 9% when Valencia orange was the pollen parent. He also found yearly variations of up to 37% with the same parents.

Overcoming Apomixis in Breeding

Gmma irradiation has been used to reduce polyembryony in *Citrus* (Speigel-Roy et al., 1972; Ikeda, 1981; Watanabe, 1985a) and in *Fortunella* (Watanabe, 1985b). Gibberellic acid treatment 30 days after anthesis has been reported to suppress polyembryony and enhance the production of zygotic progeny (De Lange et al., 1977; Mohamed et al., 1978).

Bud pollinations (substantially before anthesis) are sometimes successful in increasing zygotic progeny. Pollination of "off season" bloom has been used successfully to obtain

hybrid progeny when normal season pollinations have failed (Barrett, unpublished).

Experienced citrus breeders can use morphological traits to separate nucellar and zygotic seedlings in many progenies. However, genetic markers are not always available and isozyme comparisons can be used successfully in some cases (Moore et al., 1988). Although isozyme analyses have failed to separate cultivars of oranges and cultivars of grapefruit, they can be used to identify zygotic seedlings. Isozymes have been used to successfully identify zygotic seedlings of Swingle citrumelo rootstock in nursery and field populations (Anderson et al.,1991).

Genetics of Apomixis

Few inheritance studies have been reported in citrus due to the long juvenility period (7 to 10 years). Parlevliet et al. (1959) reported that crosses of monoembryonic X monoembryonic parents produced only monoembryonic offspring. Crosses between monoembryonic and polyembryonic parents produced a wide range of ratios but often approach 1:1. Polyembryonic X polyembryonic parents usually produce progeny with polyembryonic seeds but there are exceptions. They proposed that a principal dominant gene (P) controls the occurrence of polyembryony, and in monoembryonic individuals the dominant allele is absent. Iwamasa et al. (1967) proposed that some parental types may have duplicate genes or modifying genes for polyembryony which complicate ratios.

Benefits of Apomixis

Apomixis (nucellar embryony) is a major obstacle to citrus variety improvement but it can also be an asset. Nucellar embryony makes possible the use of heterosis with limited seedling variation in rootstock propagation by seed, the most economical method. Also, plant pathologists have found that viruses seldom are transmitted through seeds, thereby eliminating diseases. Production of nucellar seedlings rejuvenates old nonvigorous citrus clones as viruses are eliminated.

Apomixis in Cell/Tissue Culture

Nucellar-derived *Citrus* callus is highly embryogenic (Spiegel-Roy and Vardi, 1984) and has been the basis for all genetic manipulations in citrus. For example, over 30 interspecific and intergeneric hybrids have been produced by protoplast fusion (Grosser, 1991; Grosser and Gmitter, 1990) and genetic transformation has been demonstrated in C. sinensis (sweet orange) (Tetsushi et al., 1990) and C. jambhiri Lush (Rough Lemon) (Vardi et al., 1990). One limitation is that nucellar embryogenic callus is difficult or impossible to obtain from some types (e.g., mandarins, trifoliate orange [Poncirus trifoliata], or trifoliate orange hybrids).

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Important Reproductive Angiosperm Mutants, and A Detailed Discussion of the *Semigamy* Mutant of Cotton

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Many of the quantum leaps in crop breeding have resulted from manipulations and modifications of the reproductive system. Some of these, such as usage of hybridization and(or) inbreeding, are widely applied, but many others are applied in relatively crop-specific manners to facilitate hybridization, inbreeding, or germplasm usage. Examples include haploidization; modified chromosome pairing and recombination; 2n gametes formation; cytoplasmic, cytoplasmic-nuclear, and nuclear male sterilization; genetic and chemical modification of sex expression and self-fertility. The collective economic importance of such crop-specific reproductive manipulations is huge, especially if considered cumulatively over time. Given ample research support, other equally profound applications will result.

Unfortunately, the importance of the reproductive system to breeding, production and productivity is not widely enough appreciated, so reproductive research has been grossly underfunded. This is rather amazing, since the reproductive system most directly affects seed and fruit yields, which, for many crops, are the item of commerce; and even for those where it is not, seed production determines the ease with which commercial seed can be produced, and the ease with which germplasm can be used for genetic improvement. Longstanding funding limitations have been exacerbated by recent funding patterns, in which the emphasis on biotechnologies has diverted and thus further depleted funding for research on organismal, genetic, and breeding-related aspects of plant reproduction.

The organizers of this workshop are to be highly commended for recognizing the massive need for research on plant reproduction, or at least apomixis, and, more specifically, for taking the seminal step towards developing a long-overdue research thrust at national and, hopefully, international levels. If we are able to render sexual crops into apomictic ones, the ensuing agricultural revolution will likely dwarf the "green revolution".

I submit that the design of a national or international apomixis research program should be comprehensive enough encompass relevant aspects of meiosis, gametophyte development, fertilization, endosperm development, and embryo development. The literature provides ample evidence that these processes are under very specific genetic control, and they are highly pertinent to apomictic systems.

One must remain cognizant of the fact that knowledge of plant reproductive processes is incredibly primitive. Intensive research is needed. More reproductive mutants need to be identified and characterized, to facilitate both the analysis andmanipulation of the reproductive system. Molecular technologies need to be integrated for analysis, engineering, and transformation. Increased knowledge of plant reproduction is likely to be needed if we are to transfer or construct optimal apomictic systems in a wide variety of economically important plants. Thus, the objectives of a well designed apomixis research program should not only embrace the near term goal of transferring certain well-recognized forms of recurrent apomixis to economically important plants, but should encourage the acquisition of new knowledge and genetic tools for manipulating the plant reproductive system.

The remainder of my portion of this presentation is devoted to exemplifying some of the pertinent reproductive mutants currently known, and secondly to discussing our work on semigamous apomixis in cotton. Dr. Crane will then present a related recommendation, which suggests that an apomixis research program should include research on certain "ideal" apomictic systems that exist in species unrelated to crop plants. The ideal systems seem to offer forms of apomixis that would be agriculturally superior to those found in crop plants and crop relatives. They may also be useful as model systems.

If we consider a few of the specific reproductive phenomena and mutants that have been recognized in angiosperms (Table 1), it is apparent that genetic regulation of the reproductive system is phenomenally specific and comprehensive. "Switches" can be eliminated or modified, either through removal or changes in expression that are presumably qualitative, quantitative, temporal, and(or) spatial in nature. A number of such abnormalities contribute to understanding of apomixis, and may help us engineer improvements or *de novo* synthesis of apomixis.

One must keep in mind that similar mutants should be obtainable in virtually all species. Furthermore, there is little doubt that more types of mutants remain undiscovered. A sustained and systematic effort to recover and characterize reproductive mutants should be undertaken by one or more accomplished cytogeneticists. Furthermore, those mutants which have been obtained need to be maintained and, where appropriate, more fully characterized. To define the

Table 1. Examples of Reproductive Mutants of Various Angiosperms That Affect Specific Stages or Structures During or After Meiosis.

MEIOSIS

OCCURRENCE: *am*

FIRST DIVISION
- **Occurrence:** *afd*
- **Chromosome behavior:** *as, ds, ... sy, ph1, pc*
- **Spindle organization:** *dv, m*

SECOND DIVISION:
- **Occurrence:** pc_1, pc_2/male, *dy, tri, jp, el, va*
- **Spindle orientations:** *ps, rp, cc*

CYTOKINESIS: *ms1*/male, *jp, dy*, pc_1, pc_2, *el, va*

PRODUCTS: *dy*, pc_1, pc_2/male (/female), *tri, el, ps* (+/- *sy*), *rp, cc, dv*

POST-MEIOSIS

MEGAGAMETOPHYTES
- **Number and origin:** aposporic mutants
- **Divisions:** *ig, ms1, jp*

MICROGAMETOPHYTES
- **Divisions and(or) sperm function:** pollinator effects

FERTILIZATION OF EGG CELL
- **Syngamy:** *hap*, pollinator effects
- **Synkaryon formation:** *Se*

ENDOSPERM DEVELOPMENT
- EBN, etc.: interspecific variation

EMBRYO DEVELOPMENT
- Polyembryony, *Se* (semigamous), chromosome elimination

temporal and sequential inter-dependencies of meiotic and post-meiotic events should be a major goal of future apomictic research, since these are very poorly understood, at best. Important alleles should be cloned, for research and possible genetic engineering. Alleles exhibiting recessiveness or dominance will be useful for basic research, whereas those exhibiting dominant sporophytic effects, or gametophytic expression are most likely to be of direct usefulness for rendering transgenic plants apomictic.

Background on *Se*-conferred Apomixis in Cotton

We are attempting to tackle some of these types of issues with respect to the *Semigamy* (*Se*) mutant of cotton, *Gossypium barbadense* (L.), which was originally reported by Turcotte and Feaster (1963).

Their subsequent work was largely summarized in 1974, at the Guelph symposium on Haploids in Higher Plants (Turcotte and Feaster, 1974). The *Se* mutant exhibited a number of intriguing and unique characteristics. Semigamous apomixis was inferred, because seed were monoembryonic, but nevertheless included normal tetraploid progenies, maternal and paternal haploids (diploid), and chimeras with maternal haploid, paternal haploid, and(or) 4x sectors. Glanding and virescence mutants were used to detect chimerism. Frequencies of the unusual progeny sometimes ranged up to 60%. Incomplete dominance was inferred from crossing data. Expression occurred when *Se* was present in the female parent, but paternal influences were clearly evident, too. The mutant was used to produce a couple hundred of doubled haploids, which performed similar to other genotypes in terms of agronomic performance. Since that time, the trait has been transferred to a number of about 10 other cytoplasmic backgrounds (Mahill et al., 1983; Stewart, pers. comm.), thus permitting nuclear-cytoplasmic substitutions via paternal doubled haploid extraction. I began working with the mutant in 1983, results of some of which I would like to briefly describe now.

Frequencies of Semigamously Derived Progeny According to Seed wWeight

By separating bulk seed lots into weight classes, we were able to show that chimeric and especially haploid seed were lighter than normal seed. Most occurred in the lower half of the seed weight distributions.

Inheritance and Gene Location

Through a series of tests involving monosomics and monotelodisomics of *G. hirsutum*, we too obtained results indicative of monogenic inheritance involving either a sporophytic gene with dominant or additive effects, or gametophytic gene action. Monosomic tests indicated that *Se* is located in chromosome 4. Monotelodisomic tests, which included both monotelodisomics for chromosome 4

indicated that *Se* is located in the short arm of chromosome 4. This arm is thought to be about 40 map units long, based on cytogenetic data; only one other gene locus, the *ml* locus, is currently known to reside in this arm, at about 23 cM from the centromere.

Time and Mode of Gene Action

A gene that is transcribed at the gametophytic stage will exhibit a pattern of expression and inheritance that mimics that of a sporophytically transcribed gene with co-dominant gene action. The data of Turcotte and Feaster did not discriminate between these possibilities. I considered it possible, if not likely, that *Se*, which affects fertilization, might be a gametophytically transcribed gene. I thus designed a test to address this issue, based on multiple regression. The design is similar to other types of so-called "joint-scaling" tests, including the more well known "generation means analyses". The design is relatively simple, involving a 3x3 factorial of genetically marked parents differing in genotype at the *Se* locus. Analysis involves regressing observed frequencies of semigamously derived progenies onto coefficients related to specific genetic models of the time and mode of gene action. Several analysis can be made, depending on one's utilization of the degrees of freedom to test specific possible source effects, and their interactions. All models, however, indicated that maternal dominance effects were most important and highly significant. Paternal effects were second most important, and moderately significant. The results indicate that the *Se* locus is transcribed prior spore formation, e.g. in meiocytes. An alternative possibility is that *Se* is transcribed in somatic tissue, but somehow brings about the semigamous behavior of gametophytes or gametes.

Development of a System for Mass-extraction of Doubled Haploids

A facile, inexpensive means for mass-extracting doubled haploids would be welcomed in diploid and disomic polyploid crops. Breeders, basic geneticists, and other biologists whose work is complicated by confounding effects of genotypic variation obviously desire genetically uniform materials, as are made possible by doubled- haploid extraction. Breeding research would benefit directly, and also indirectly, since the knowledge base provided by more fundamental studies would be superior. Testing of genotypes is the most resource- consuming part of plant breeding. Inbreeding is not especially expensive, but it does require time. To the extent that accelerated inbreeding does not detract from efficacy of a breeding program's overall sequence of selection, development of a facile, cheap means for rapid inbreeding can accelerate the rate of genetic improvement. We are exploring the development of a highly efficient system of mass-extraction of doubled haploids of cotton.

The system is denoted by the acronym HEHP ("hep"), which stands for **H**ybrid-**E**liminating **H**aploid-**P**roducing. The HEHP system includes two major components, which correspond to the name. A complementary hybrid lethality system is used to eliminate all tetraploid hybrid progenies, whereas the *Semigamy* mutant is used to produce the haploids, i.e. the source of the doubled haploids. To apply the HEHP method, a HEHP parent will be crossed extensively with another parent, from which doubled haploids are desired. After mass-treatment to induce chromosome doubling, the seed can be sown at very high densities, because all but the haploids and doubled haploids will perish. Fertility and markers will distinguish haploid *vs.* doubled haploid progenies, and haploid progeny/sectors of maternal vs. paternal origin. For instance, we are using the dominant male sterility allele Ms_4 to develop HEHP populations amenable to efficient cross-pollination. Also, we have developed an excellent anthocyanin-containing (R_1) semigamous line that is being integrated into our HEHP breeding efforts. The anthocyanin is an excellent seedling and plant marker, and is ideally suited to the practical usage of the HEHP system. In other works, we want genetic tools to do most of the work, and facilitate the rest of it. Being simple and cheap, the method should be of interest to most basic and applied scientists and breeders. The hybrid lethality system being used is conferred by alleles of the Le_1 and Le_2 loci. The allele Le_2^{dav}, which was extracted from a wild Mexican species, interacts with alleles Le_1 and(or) Le_2 to cause lethal necrosis at during seed development or seedling stages. Le_2^{dav}, Le_1 and Le_2 alleles, however, are completely compatible with alleles Le_1 and le_2, which also are completely compatible with each other. Virtually all tetraploid cottons are homozygous for Le_1 and Le_2. HEHP stocks will carry both *Se* and Le_2^{dav}. The former causes the needed reproductive abnormalities, whereas the latter.eliminates normal 4x hybrid progeny arising from

Figure 1.

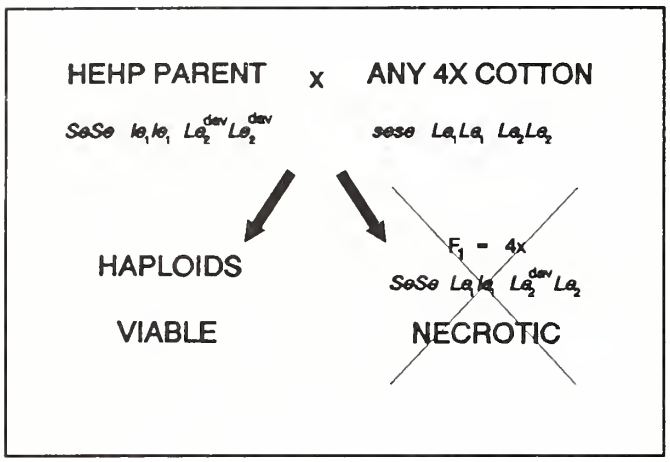
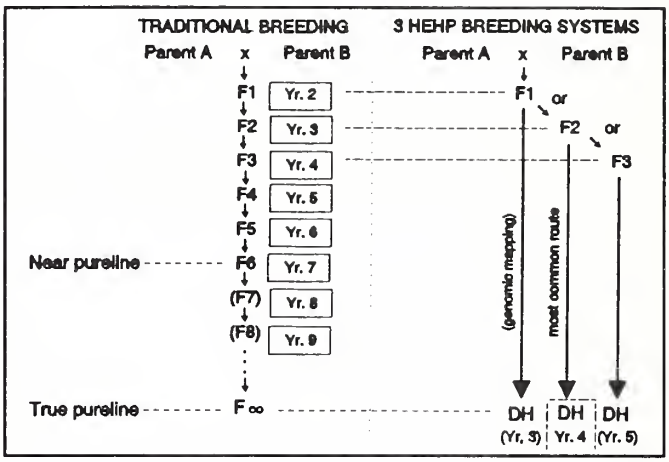


Figure 2.



crosses with Le_1Le_2 homozygotes. Yet, HEHP stocks will be fully fertile, except as modified to facilitate crossing, e.g. by inclusion of male sterility gene(s), so maintenance of HEHP should require no special procedures.

If the HEHP system works well, it will allow the user to apply it to virtually any material from which doubled haploids are sought (Figure 2). For example, the genomic mapper would probably apply it to extract doubled haploids of F_1 hybrids, giving nearly immediate access to replicated field testing. Researchers and breeders could use it to purify stocks and render them true-breeding. Breeders might most often apply it to F_2 generations, i.e. following mass-selection for highly heritable traits, or possibly to F_3 generations, i.e. following selection for traits that are selectable on the basis of progeny-rows or limited replications or sites. Preliminary tests have demonstrated that the HEHP system does lead to the selective recovery of haploids. Tests dedicated to recovery of double haploids, i.e. involving a chromosome-doubling technique, have not been completed to date.

Emphasis of Current Research Pertaining to *Se*

Our present work on *Se* emphasizing four areas: [1] development of HEHP parents, and related techniques; [2] analysis of environmental influences of the frequency of semigamous reproduction; and [3] cytology and ultrastructure. Lastly, we are mapping the *Se* locus relative to the centromere and ml; developing materials for locating and determining linkages between *Se* and molecular markers (RFLPs and RAPDs), that will be used for map-based cloning or YAC-based cloning. An RFLP map is being developed at this time, as is a YAC library.

While the *Se* mutant is of value in itself, both biologically and as a breeding tool, it should also serve the a useful role in the overall apomixis research program. It is likely that it specifically affects the mechanism that brings the sperm and egg nuclei together, and(or) their fusion. Thus, it probably is the only mutant known which is likely to specifically affect this event in plants.

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A Rationale for the Investigation of Certain Wild Apomicts

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Although several distinct types of apomixis have been discussed at this workshop, there exist other apomictic systems as well. Some of them might outperform the *Panicum* and *Elymus* systems upon transfer to crops by genetic engineering, while others offer considerable advantages in genetic or molecular-developmental studies that could lead to the identification and cloning of apomictic genes.

The first slide lists some characteristics of an ideal apomictic system for crop production. Given the objective of stabilizing elite hybrid lines, the need for obligacy and freedom from subsexual variation seems obvious. Two sources of subsexual variation will be mentioned on a subsequent slide. High seed fertility is obviously important in grain crops; increased seed fertility has also been long sought in forage apomicts like buffel- grass. Normal pollen and simple inheritance are needed to ease the synthesis of new apomictic varieties, thus decreasing the temptation to release very few apomictic lines that end up covering huge areas with genetic vulnerability. Diploid expressivity likewise is needed for efficient breeding; some crops might decrease in vigor or fertility as induced polyploids, even if obligately apomictic.

The second slide lists additional characters in an optimal research apomict. Ease of embryological examination and precise control of crosses are obvious aids in light of experience in various grasses. Large progeny size increases the statistical robustness of proposed dominance relations and numbers of loci responsible for apomixis. Tight correlation of ovule to bud or anther development permits the timing studies (like Crane and Carman, 1987) that increase our understanding of parallel developmental pathways and of the fundamental nature of each type of apomeiosis. Perenniality permits conserva- tion of genetic stocks and crosses with earlier generations, while quickness to flower lets the worker keep up with the demands of modern publication, or at least complete the study within his lifetime. Availability of interfertile sexual and apomictic forms, without hybrid breakdown or irregular segregation in subsequent generations, is critical to classical genetic studies of apomixis. Such availability rarely can be taken for granted, as apomicts tend to displace or genetically assimilate their sexual forbears. An abundance of easily scored seedling markers greatly reduces the work in progeny tests. Ease of transformation, implying ease of regeneration from tissue culture, is essential if the functions of various cloned genes or antisense constructs to cDNA's are to be understood.

The third slide illustrates some problems with apomictic systems discussed elsewhere in this workshop. This hardly means that these systems should be abandoned, only that some effort should be devoted to alternatives that offer improvement. As much as I like grasses, I find them difficult to emasculate in comparison to two-inch lily buds, as occur in *Habranthus* and *Zephyranthes*. Some panicoids have dark purple pigments in the ovary wall that interfere with clearing, while *Elymus* and most Asteraceae contain birefringent calcium oxalate crystals that wreak havoc with Nomarski microscopy. The apomeiotic mechanism of *Elymus* rectisetus includes a preapomeiotic episode of nuclear stretching that can cause chromosome breaks, leading to translocation upon reunion or monosomy upon loss of a centromere. Apomixis in *E. rectisetus* is correlated with low pollen and seed fertility, although there is considerable variation among accessions.

The *Panicum* system includes the induction of multiple embryo sacs from nucellar cells. These embryo sacs compete with one another and, in some species, with the sexual embryo sac. When none of the embryo sacs matures properly, seed set is reduced, while at other times two or more embryo sacs function in the same ovule, resulting in polyembryony. Even the *Rubus* scheme, which occurs in many species of *Poa*, often initiates more than one embryo sac per ovule.

The meiotic restitution of *Taraxacum* can lead to two types of instability. In *Rudbeckia sullivantii* (Battaglia, 1955), limited crossing over occurs in prophase I, with chromosome associations up to quadrivalents. Genes distal to these crossovers are homozygosed, eventually leading to inbreeding depression even in an obligately apomictic strain. The asynapsis or desynapsis of apomictic *Taraxacum* suppresses such recombina- tion, but usually at the price of affecting the pollen development as well, which renders the apomict less genetically accessible in a breeding program. (The problem is not insurmountable in *Taraxacum*, in that pollen from triploid apomicts can cross to sexual diploids to give novel triploid and tetraploid apomicts.) A second source of subsexual variation in apomictic *Taraxacum* is occasional exclusion of univalent chromosomes from the first-meiotic restitution nucleus, leading to "monosomics" (usually 3x-1).

The fourth slide mentions three apomicts that improve on the characteristics of *Panicum maximum*, *Elymus rectisetus*, and *Taraxacum* officinale. The three are Habran- thus *robustus*, *Zephyranthes pulchella*, and *Blumea eriantha*. While none of them is perfect, their advantages are clear. The first two are little lilies (Amaryllidaceae) whose flowers are easily manipulated and whose ovules are benchmarks for clearing in methyl salicylate. The *Habranthus* has up to 150 ovules per flower, is diploid, and is amenable to chromosomal in situ hybridization. Unfortunately, no conspecific source of sexuality is known, although *H. cardenasianus* and probably *H. immaculatus* are sexual. Also, the buds differentiate inside the bulb, making the stages of semigamy much more accessible than the apomeiosis. The latter appears to be strictly mitotic, without potential for subsexual variation, but this needs to be checked critically. The generation time is also a bit long (13-16 months to first flowering), though short for amaryllids.

Zephyranthes pulchella contains sexual tetraploid and apomictic octoploid popula- tions. The sexual is an endemic on the verge of extinction because of urbanization around Soto la Marina, Tamaulipas, Mexico. Chromosome doubling of the tetraploid, or backcrossing of the (4x x 8x) F1 with the 4x, should give an 8x stock that would form arbitrarily large numbers of segregating hybrids with the 8x apomict. Interspecific and even intergeneric hybrids of *Z. pulchella* tend to be highly fertile; the apomictic hybrid with *Cooperia drummondii* is known as *C. smallii* and has 90 - 98% stainable pollen. The problems are unknown pattern of genomic affinity in the octoploid, which is likely close to autooctoploid, and a full two-year generation time.

Blumea eriantha appears to be one of the few apomicts that is obligate for the second meiotic division without evidence of the first (Chennaveeraiah and Patil,1971), i.e. there is no restitution of a first prophase or metaphase and thus no recombination or univalent exclusion. This form of apomeiosis would be excellent if it could be genetically engineered into a crop species.

The fifth slide brings up a second rationale for investigating certain wild apomicts. We do not yet know how conserved the genetic controls of megasporogenesis are. The presence of genic seed sterility in some interspecific hybrids suggests that some of the regulatory genes evolve rapidly. If this is the case for gene(s) that cause apomixis, genetic engineering of them will yield functioning apomixis only if the donor and recipient are relatively closely related. The slide lists 18 crops that have known or putatively apomictic relatives. Double question marks signify donors suspected of apomixis on the basis of odd polyploidy, aneuploidy, or very high polyploidy. The Malvaceae (see *Hibiscus*) have embryologically unknown high polyploids in several genera. There is a need for recon- naissance of such groups before narrowly endemic sexual forms of otherwise apomictic species are wiped out by habitat destruction.

The sixth slide provides a summary: Genetic and molecular-developmental investigation of wild apomicts is justified because some have superior apomictic mecha- nisms, because critical sexual relatives are now succumbing to development of the tropics, and because taxonomic variation in embryological regulation might limit the range of genetic engineering of apomixis.

An Explanation for the Genetic Control of Apomixis In *Cenchrus*

I have a pet explanation for the segregation patterns obtained in *Cenchrus ciliaris* by Taliaferro and Bashaw (1966). This explanation involves preferential segregation among three alleles in a partially tetrasomic tetraploid; the pattern of preferential segregation varies among the three genotypes: SB, sexual buffelgrass; CB, common buffelgrass; and BB, blue buffelgrass. The alleles are a (wild-type sexual), A (quasidikary- onic, = aposporous in the terminology of Gustafsson, 1946), and A° (super-sexual mutant). Genotypes AAAA, AAAa, and AAaa are apomictic; all others are sexual, i.e., one dose of A is not enough and one A° overwhelms three doses of A.

There are probably several plausible biochemical genetic explanations for the dominance relationships among A°, A, and a. For one example, consider the A gene product to be a negative regulatory protein that binds upstream from a coding region whose product somehow induces apospory (somatic quasidikaryony). The products of A°, A, and a differ in their affinity for the promoter or nearby site, and in their effectiveness in shutting off all transcription from the open reading frame. The product of allele a binds as tightly as the product of A, and shuts the transcription off more completely. The product of A° is as effective as the product of a, but it binds to the DNA more tightly. Thus, even if

only one copy of A° is present, its product will tend to occupy all the promoter or nearby sites, thereby shutting off all copies of the downstream gene and permitting sexuality.

For the following calculation of skewed tetrasomic segregation, the A locus is presumed to be tightly linked to the centromere, although the model probably could be expanded to include double reduction without loss of fit. Let us number the four chromosomes of a homologous group 1, 2, 3, and 4. Regardless of the patterns of pachytene association or anaphase disjunction, all meioses that yield euploid gametes will do so in one of three patterns: 1 and 2 in one meiotic product (pair of microspores or megaspores) and 3 and 4 in the other, or 1 and 3 in one spore pair and 2 and 4 in the other, or 1 and 4 in one spore pair and 2 and 3 in the other. In the seed parent, let r_1 be the probability of a 12 + 34 outcome, r_2 be the probability of 13 + 24, and r_3 be the probability of 14 + 23, such that $r_1 + r_2 + r_3 = 1$. We can then give the following probabili- ties of each kind of egg cell:

x_1 = probability of 12 = $r_1/2$

x_2 = " 13 = $r_2/2$

x_3 = " 14 = $r_3/2$

x_4 = " 23 = $r_3/2$

x_5 = " 24 = $r_2/2$

x_6 = " 34 = $r_1/2$.

Analogous probabilities of microspore pairs exist in the pollen parent. Let them be designated s1 for the pair 12 + 34, s_2 for 13 + 24, and s_3 for 14 + 23, yielding pollen gametic probabilities of:

$y_1 = s_1/2$ for 12

$y_2 = s_2/2$ for 13

$y_3 = s_3/2$ for 14

$y_4 = s_3/2$ for 23

$y_5 = s_2/2$ for 24

$y_6 = s_1/2$ for 34.

These probabilities can be used to generate a Punnett square with six x columns (x_1 to x_6) and six y rows (y_1 to y_6). The element at the ith column and jth row is thus $x_i y_j$. One need merely sum the appropriate cells of the Punnett square and multiply by the total progeny size to obtain the expected numbers of sexual and apomictic offspring, which can be tested against the observed totals with the chi-square test. I have written a simple, ad-hoc Fortran program to do this, given the following genotypes, where each subscript designates the chromosome that bears the allele within the homologous group:

$SB = A^\circ_1 A_2 a_3 a_4$

$CB = A_1 A_2 a_3 a_4$

$BB = A_1 A_2 A_3 a_4$

Thus the probability of an A°A egg from SB is x_1, an aa sperm is y_6, and so on. Note that the 13 + 24 and 14 + 23 arrangements in SB both give one A°a and one Aa, so that the values of x_2, x_3, x_4, and x_5, do not matter, so long as they sum to $1\text{-}r_1$ and $x_2 = x_5$ and $x_3 = x_4$. The predicted egg segregation in SB depends only on r_1, and analogously the predicted pollen segregation in SB depends only on s_1. The same is true in CB: only the 12 + 34 pattern gives gametes that differ. In BB, half the gametes will be AA and half Aa, regardless of the pattern of genomic affinity. Thus I needed only to optimize r_1 (= s_1) in SB selfed, and use that value of

r_1 in finding the best value of s_1 in SB x CB. Finally, I used the same r_1 for SB x BB and hoped that it gave a reasonably close fit. Values of r_1 and s_1 were optimized by trial and error, observing which direction of change improved fit. Here are the results:

Cross	r_1	s_1	Observed	Expected	X^2
SB selfed	0.2051	0.2051	S: 517 A: 97	S: 517.009 A: 96.991	0.00000
SB x CB	0.2051	0.3130	S: 373 A: 202	S: 373.004 A: 201.996	0.00000
SB x BB	0.2051	any	S: 108 A: 83	S: 105.294 A: 85.706	0.15503

None of the chi-squared values indicates a significant difference of expected from observed frequencies. The low value of r_1 in SB indicates that chromosomes bearing A' and A tend to pair at meiosis, with each allele going preferentially to a different gamete. The value of s1 in CB is consistent with random pairing and segregation. Although chromosomes form bivalents more often than expected for random association in buffelgrass, their small size or other genetic influences might reduce the independence of their arms in pairing, or increase the range of chiasma interference.

I hope that future genetic investigations of apomixis will pay similar attention to the possibilities of multiple allelism and preferential segregation. The model here can be easily adjusted to estimate preferentiality in a duplex diallelic heterozygote for any codominant marker. The values of r_1, r_2, s_1, and s_2, thus obtained can constrain the values for predicting sexual:apomict segregation.

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Apomixis in *Bothriochloa*, *Capillipedium*, and *Dichanthium*

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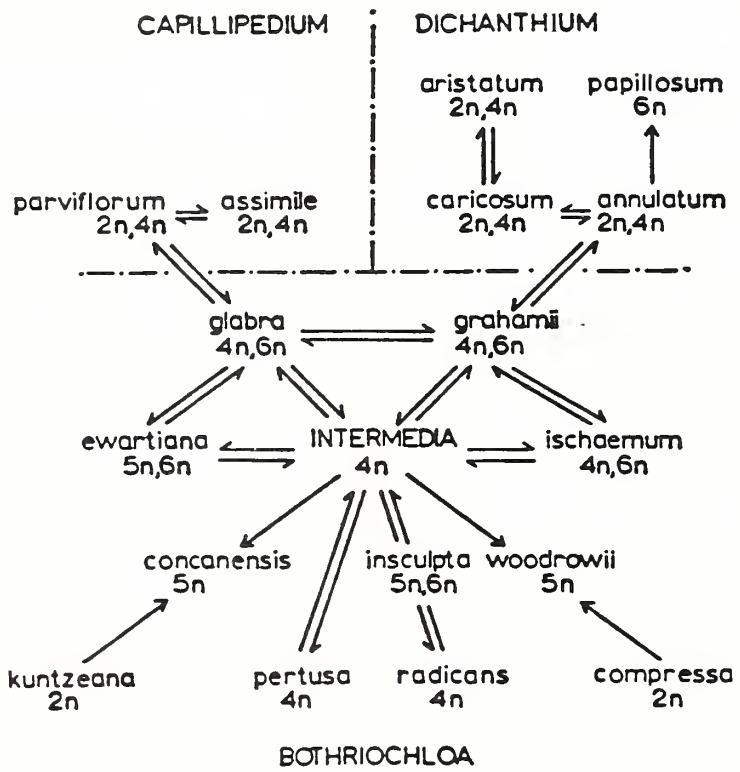
The information which I present here will, for the most part, be a summary of work conducted by J. R. Harlan, J.M.J. de Wet, and their students and colleagues from about 1950 to 1970. During this time, they assembled a large collection of the old world bluestem (OWB) grasses at the Oklahoma State University and conducted comprehensive biosystematic investigations of the complex.

The genera *Bothriochloa* O. Kuntze, *Dichanthium* Willemet, and *Capillipedium* Stapf comprise a classic agamic complex in which diploids (x=10) are sexual; tetraploids are usually, but not always, apomictic; and higher ploidy levels are obligate apomicts, or nearly so (Harlan & Celarier, 1961). Diploid species and species with high ploidy levels (>6X) tend to have relatively restricted distributions and are often found as narrow endemics; tetraploids are most common and widespread (Harlan and de Wet, 1963). The genera are genetically united via the bridging compilospecies *B. bladhii* (Retzius) Blake [=*B. intermedia* (R. Brown) A. Camus] depicted by de Wet and Harlan (1969) as follows:



Harlan and de Wet (1963b) describe a variety of cytogenetic mechanisms within the OWB complex that operate to produce a labile, dynamic, highly adaptable system capable of a high degree of adaptive polymorphism and rapid evolution. These include mechanisms that:

1) promote contact with sexuality,

a. doubling the chromosome number of a sexual diploid
b. functioning of unreduced eggs of sexual tetraploids
c. maintenance of facultative races
d. crossing of facultative races and temporary recovery of sexuality
e. functioning pollen of obligate apomicts
f. production of sexual embryo sacs in apomicts, and
g. promote cross-fertilization

2) increase heterozygosity and heterosis

a. functioning of unreduced gametes
b. preferential chromosome pairing
c. gene control of chromosome pairing, and
d. apomixis

3) promote variability and adaptive polymorphism.

1. mutation
2. autoploidy
3. parthenogenetic development of reduced eggs, and
4. introgressive hybridization

Central among these mechanisms is apomixis which permits escape from sterility in hybrid complexes and thereby provides a means to rapidly colonize available habitats with superior genotypes.

The apomictic mechanisms in OWB's are well defined; but genetic control of the process is yet to be elucidated (Celarier and Harlan, 1957; Harlan and de Wet, 1963b; Harlan et al., 1964; de Wet and Harlan, 1969; Saran and de Wet, 1970). Apomixis in OWB is by pseudogamous apospory with unreduced embryo sacs arising from the nucellar tissue of the ovule. Aposporous embryo sacs are characterized as having a single large polar nucleus, an egg, two synergids, and no antipodals. The number of aposporous embryo sacs is genotype dependent and may range on average from one to several. All apomicts in the species apparently have some sexual potential, of which only a part or none may be realized (Harlan et al., 1964; de Wet and Harlan, 1969). Apomictic and sexual embryo sacs are always produced, side-by-side, even in the most apomictic biotypes of the agamospecies. Depending on genotype and the environment, either one of these embryo sacs may develop embryos parthenogenetically, or become fertilized and develop sexually.

The overall apomictic mode of reproduction in OWB's is inherited as a dominant character over sexuality. The degree of sexuality of an apomictic plant is characterized by Harlan et al. (1964) as being dependent upon the synchronization of various embryological phenomena, almost certainly under complex genetic control. However, they stated that apomixis, or lack of it, is rather simply inherited and is controlled by no more than one gene per genome. They describe apomixis and sexuality as not being alternative reproductive modes, either genetically or operationally, but simultaneous and independent phenomena. They further express the opinion that the genes controlling normal sexual reproduction are not allelic to those controlling apomixis in the conventional sense. In their paper, the following genetic mechanism is hypothesized: In apomicts, the genotype Aa may be assigned in the sense that the A allele induces four-nucleate embryo sac formation and the a allele does not. Autonomous parthenogenetic embryo development in reduced sacs may occur independently of the primary apomictic mechanism. When both kinds of sacs are produced, however, parthenogenesis tends to induce precocious embryos in sexual sacs, thereby reducing the realization of sexual potential. Under this scenario, therefore, three independent phenomena may operate simultaneously: normal sexual reproduction, parthenogenetic autonomous development of embryos, and nucellar apospory. They theorized that tetraploid plants could be assigned the genotype AaAa and the sexual ones the genotype aaaa. However, all of the tetraploid apomicts which they studied were demonstrated to be heterozygous for genes conditioning sexuality, leading them to conclude that some sort of balanced heterozygous system may be involved. Such a balanced heterozygous system was demonstrated by Nogler (1984) in *Ranunculus auricomus* L. where the gene(s) for apospory can only be transmitted in heterozygous diploid or polyploid gametes. In haploid gametes or homozygous polyploid ones, the gene is apparently lethal. Kellogg (1990) notes that if this is more generally true, then it would help to explain the correlation of agamospermy with polyploidy, as well as the fact that crosses of agamospermous parents give rise to sexual offspring.

That the overall mechanism of apomixis versus the absence of it in OWB's is relatively simply inherited is according to Harlan et al. to be expected. They base this conclusion on the fact that apomixis is widespread in the complex and therefore it either evolved over and over on different occasions in different branches or it spread by genetic means after having been established. They believe the latter is the far more likely alternative.

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Future Research

At the conclusion of the Apomixis Workshop the attendees were asked to respond to the following questions in an effort to develop an overall statement of future apomixis research needs.

I. What is the general goal of apomixis research?

II. What are the separate objectives to reach this goal?

III. How do we do it?

IV. Estimate time to reach the goal?

The group answered these questions as follows:

I. The general goal of apomixis research is to be able at will to isolate and transfer (move) the genes that control apomixis into important agronomic and horticultural species. Of greatest significance would be transfer of apomixis into high performance hybrids that presently require reconstituion at great cost from their two individual parents at each generation planting. Apomictic hybrids could be multiplied (cloned) continually through seed produced by the hybrid for inumerable seed generations.

II. The separate objectives to reach this goal are:

   a) Develop better understanding of apomictic process (in species in which apomixis presently exists)—genes involved, gene action, pathways, processes, etc.

   b) Tag and isolate genes controlling apomixis—molecular markers, cytology, other systems for gene identification.

   c) Transfer genes controlling apomixis to target plant species develop transfer systems, molecular methods, conventional breeding.

III. In order to reach these objectives we:

   a) Develop an ARS and SAES organizational structure identifying locations, species to be researched, and specific research objectives for each component of the organization. Coordination of research across the research locations will be essential for most efficient and effective research.

   b) Develop multi-location teams composed of ARS and SAES researchers.

   c) Foster communication among the apomixis researchers.

   d) Provide motivation for apomixis reserach through funding thrusts specifically designated for this research. Other possible sources of funding would be the NRI, and industry cooperative funding grants.

   e) The time requirement to reach the overall goal will likely be 20+ years, however, numerous individual goals relative to the separate objectives listed in II above will be attained within 5-10 years for some and within 10-20 years for others.

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